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A NEW METHOD FOR DETERMINING THE POROSITY OF THE SOIL

G. TORSTENSSON AND SIGURD ERIKSSON

Agriculture College of Sweden, Uppsala

Received for publication August 18, 1936

In the methods for determining the porosity of the soil worked out by Kopecky (3), Burger (2), et al., a fixed volume of soil is divided into soil substance, soil water, and soil air. The volume of the whole sample is equal to that of the cylinder used for taking the sample; the water content is found by drying the soil; and the volume of the soil substance is determined by multiplying the soil weight by the specific gravity of the soil. The air content in the soil sample will then be found by difference:

100 − (per cent soil substance (vol.) + per cent soil water (vol.)) = per cent air (vol.) in the soil.

Other principles have also been worked out, for example that of Nitzsch (16), who calculated the volume of the soil substance as the difference between the weights of the soil in air and under water, or that of Dojarenko (4), who determined the water content by shaking the soil in alcohol and measuring the soil volume directly.

These methods are laborious and, as the air content in all of them will be found by difference only, the value will be more or less doubtful when dealing with soil cultivation investigations, in which the air content is the prime factor.

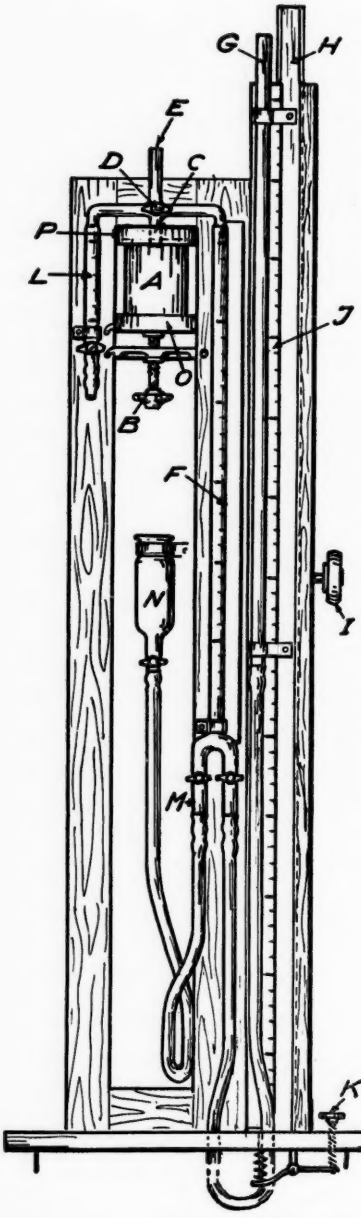
Recently we described a new method (8) by which the air porosity of the soil can be measured directly. Soon afterward, Nitzsch (7) also brought out a similar method.

In our method, rustless steel cylinders are used for taking the soil sample in the same way as that used by Burger (2), Blohm (1), Nitzsch (6) et al., but for determining the air porosity a special apparatus, "the porosimeter," is used.

DESCRIPTION OF APPARATUS

The soil samples are taken, in their natural condition, with the aforementioned cylinders and put into cylinder *A* (fig. 1). The cylinder is standing on a plate *O* which can be moved by a screw *B* and fixed airtight to a rubber packing *P*. Through this packing a glass tube *C* passes; by means of the tap *D* the cylinder *A* can, as desired, be connected with the atmosphere *E* or with the tube system *F-G*, which is filled with mercury.

The glass tube *F*, mounted on a mirror, has a volume of 50 cc. and is divided



A Cylinder in which the soil samples are measured.
B Screw for fixing plate *O* with cylinder *A* to rubber packing *P*.
C Glass tube.
D 3-way-tap.
E Glass tube to the atmosphere.
F Glass tube divided into 0.1-cc. units.
G Glass tube on a movable sliding piece *H* connected by a rubber tube with the glass tube *F*.
I Screw to move *H*.
J Reflecting glass scale.
K Screw for adjusting *J*.
L Correction tube.
M Rubber tube to adjustment vessel *N*.

FIG. 1. DIAGRAM OF THE POROSIMETER

into 0.1-cc. units. A rubber vacuum tube connects it with the glass tube *G*, which is mounted on a sliding piece *H*. By a screw *I* this can be moved upward and downward. Behind *G* there is a scale marked on a mirror, which can be adjusted by screw *K*. The total volume of the system *L-A-F* can be corrected by means of glass tube *L* to whole cubic centimeters for easier calculation. The amount of mercury in the system *F-G* may be adjusted from the vessel *N* and the rubber tube *M*.

If we connect the tube *F*, through tap *D*, with the atmosphere, the mercury will have the same level in both tubes *F* and *G*. If, on the contrary, the tap is set to connect tube *F* with the cylinder *A*, and we move tube *G* upwards with screw *I*, we will have an excess pressure in *A* and tube *F*. This excess pressure can be read off on the scale *J*.

METHOD OF DETERMINATION

The following terms are used:

- v_a total volume of cylinder *A* and glass tube *F* to the 0-mark.
- v_j total volume of the soil (in wet samples = soil + water).
- b barometric pressure.
- t pressure difference.
- f volume variation.

When using the porosimeter, we first determine v_a. For this purpose it is necessary to begin with a relatively low reading of the mercury in tube *F*, and to reduce the air volume f cc. by moving the tube *G* upwards. The volume will then be $v_a + f$ at the beginning of the measurement, and the pressure b cm. of mercury. When the mercury in the tube *F* is at the O-mark, the air volume is v_a and the pressure $b + t$ cm. According to Boyle's law we will have the following equation:

$$(v_a + f) \cdot b = v_a (b + t)$$

then

$$v_a = \frac{bf}{t} \qquad (A)$$

If we put a soil sample in the cylinder *A* the volume v_j will be found in an analogous way

$$(v_a - v_j + f) \cdot b = (v_a - v_j) \cdot (b + t)$$

and then

$$v_j = v_a - \frac{bf}{t} \qquad (B)$$

By determinations with the porosimeter, therefore, the air volume is measured and the soil volume calculated as we have already shown.

The apparatus can also be used for determining the specific gravity of the soil; if v_j in formula (*B*) refers to dry soil and its weight is a gm. the specific gravity will be

$$s = \frac{a}{v_j}$$

If, on the contrary, the specific gravity of the soil is known, the porosimeter can be used for determining the water content of the soil. Assume a soil sample of a gm., with a specific gravity s, volume v_j cc., and p per cent water content by weight, then the volume of the water + soil substance is equal to the total volume of the wet sample according to the following equation:

$$\frac{p}{100} \cdot \frac{a}{1} + \frac{(100 - p)}{100} \cdot \frac{a}{s} = v_j$$

then

$$p = \frac{100\,(v_j s - a)}{a(s - 1)} \text{ per cent (weight)} \qquad (C)$$

PRACTICAL APPLICATION

In our earlier paper (8) we discussed the principle of the apparatus and showed its applicability. In the present paper actual figures are given for comparison of this method with existing methods. These investigations have been done on different soils, varying from strongly marked sandy and peaty to heavy clay soils.

Experiments were first conducted on a sandy soil, poor in humus, of the following texture:

	per cent
Coarse sand (2–0.2 mm.)	68.3
Fine sand + coarse silt (0.2–0.02 mm.)	19.4
Fine silt (0.02–0.002 mm.)	1.8
Clay (<0.002 mm.)	7.6
Organic matter (loss on ignition with reduction according to the clay content)	2.9

Of this soil six samples were taken from the surface layer in their natural condition, to a depth of 10 cm., with our rustless steel cylinders. The samples were first weighed, and their porosity, i.e., air space, was determined in the porosimeter.

For comparison with existing methods, the samples were then dried at 110°C., to constant weight, and weighed. The dry soil was once more tested in the porosimeter, and the specific gravity of the soil was determined according to the method of Zunker (5). The figures obtained by weighing the soil before and after drying enabled us to calculate, in the usual way, that part of the pore space which is filled with air. In table 1 the values calculated in this way are compared with those obtained in the porosimeter. In the second column the total volumes of the soil samples are tabulated, in order that the reader may

use the figures for other calculations. The absolute agreement between columns 3 and 4 will depend a little on the value of the specific gravity of the soil, which is used for calculating the figures in column 3. It can be seen that the values in column 3 are, generally, lower than those in column 4. This difference, in itself of no importance, is due to the fact that the value for specific gravity obtained in the pyknometer was a little too low, because some plant residues were floating. This is confirmed by the fact that another determination of the specific gravity on the dry soil in the porosimeter gave an average value of 2.69 from six determinations, whereas that obtained in the pyknometer

TABLE 1

Comparison of the values obtained on sandy soil in the porosimeter with those calculated in the usual way

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	PORE SPACE CALCULATED IN THE USUAL WAY	PORE SPACE FOUND IN THE POROSIMETER
	cc.	*cc.*	*cc.*
25	665	256	257
26	673	230	236
27	673	217	224
28	670	244	252
29	676	249	256
30	679	267	273

TABLE 2

Comparison of the water content of sandy soil calculated according to formula (C) *with that found by drying the sample at 110°C.*

NUMBER OF THE SAMPLE	WATER CONTENT ACCORDING TO (*C*)	WATER CONTENT DRIED AT 110°C.
	per cent	*per cent*
25	9.5	9.5
26	9.0	9.5
27	9.3	9.9
28	8.5	9.4
29	8.1	8.8
30	8.4	8.8

was 2.67. The figures in column 3 are calculated on the basis of the pyknometer value 2.67.

As an example of the application of formula (*C*), table 2 is given. If the volume of soil substance + water in the porosimeter has been found to be 407 cc. and the weight of the soil in the cylinder 943 gm., the water content in the soil will be determined as follows

$$p = \frac{100 \cdot (407 \cdot 2.69 - 943)}{943 \cdot 1.69}$$

which gives $p = 9.5$ per cent.

In this case we have used the specific gravity obtained in the porosimeter, because the intention is to use only the porosimeter.

The agreement between the values in columns 2 and 3 is, perhaps, not so good as desired, in view of their small size; but the variation in column 2 is nevertheless only slightly greater than that in column 3.

The next experiments were conducted on samples from the surface, also to a depth of 10 cm., of a much finer sandy soil, of the following texture:

	per cent
Coarse sand (2–0.2 mm.)	6.8
Fine sand + coarse silt (0.2–0.02 mm.)	75.1
Fine silt (0.02–0.002 mm.)	4.3
Clay (<0.002 mm.)	9.1
Organic substance (loss on ignition with reduction)	4.7

The determinations were the same as those described for the sandy soil. The difficulty in obtaining one value for the specific gravity, representing the

TABLE 3

Comparison of the air space, of fine sandy soil, obtained in the porosimeter with that calculated in the usual way

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	AIR SPACE CALCULATED, S. G. 2.63		AIR SPACE CALCULATED, S. G. 2.73		AIR SPACE FOUND IN THE POROSIMETER	
	cc.	*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*
31	683	178	26.1	190	27.8	186	27.2
32	684	196	28.7	207	30.3	208	30.4
33	679	206	30.3	217	32.0	208	30.6
34	682	166	24.3	177	26.0	181	26.5
35	679	174	25.6	186	27.4	192	28.3
36	679	163	24.0	174	25.6	178	26.2
Average			26.5		28.2		28.2

whole sample, was, in this case, even greater, because of an increase in the amount of plant residues in the upper part of the sample. To show the possible differences, we give three values for the air space in table 3, one of which has been calculated on the basis of specific gravity 2.63, obtained by the pyknometer method; a second, based on the value 2.73, found in the porosimeter; and a third, from direct measurement in the porosimeter. The value 2.73 is also found by solving s in the formula (C), after inserting the value for water content found by drying the soil at 110°C.

For a comparison of our method with existing methods, columns 3–4 should be compared with 7–8 (table 3). Taking into account what has already been said regarding the difficulty of obtaining a representative value for the specific gravity of the whole sample, we have found it convenient also to make the corresponding calculation in columns 5–6, using the considerably higher value found in the porosimeter. The figures in columns 5–6 and 7–8 show the best

TABLE 4

Comparison of the air space, of heavy clay, found in the porosimeter with that calculated in the usual way

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	AIR SPACE CALCULATED IN THE USUAL WAY	AIR SPACE FOUND IN THE POROSIMETER
	cc.	*cc.*	*cc.*
1	667	174	178
2	669	142	140
3	673	125	125
4	672	149	150
5	670	145	157
6	676	164	165
7	667	133	135
9	674	137	135
10	666	161	161
11	672	121	120
12	672	128	129
13	680	161	161
14	669	126	125
15	676	133	133
16	680	155	155
17	680	161	163
18	679	174	174

TABLE 5

Comparison of water content of heavy clay calculated according to (C) *with that found by drying*

NUMBER OF THE SAMPLE	WATER CONTENT, ACCORDING TO (*C*)	WATER CONTENT, DRIED AT 110°C.
	per cent	*per cent*
1	22.1	22.8
2	23.9	23.6
3	23.6	23.6
4	22.8	22.9
5	20.8	22.6
6	23.3	23.4
7	22.1	22.5
8	22.6	Spoiled
9	23.0	22.7
10	22.0	22.0
11	22.4	22.3
12	22.0	22.2
13	22.7	22.7
14	23.4	23.2
15	23.3	23.3
16	22.9	22.9
17	22.0	22.4
18	22.9	22.9

agreement and, in this case, even the same average percentage of air-filled pore space. When we are dealing with such a varying quantity as air space, however, the deviation between the different reiterations is necessarily large.

TABLE 6

Comparison of calculated air space in three different peaty soils with that found in the porosimeter

SOIL	NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	AIR SPACE CALCULATED	AIR SPACE, FOUND IN THE POROSIMETER
		cc.	*cc.*	*cc.*
Well-decomposed peat, s. g. 1.81	1	667	166	162
	2	669	148	145
	3	673	164	161
	4	672	164	162
	5	670	175	172
	6	676	184	183
Moderately decomposed peat, s. g. 1.73	7	667	97	97
	8	678	102	103
	9	674	115	113
	10	666	122	119
	11	672	157	156
	12	672	162	159
Slightly decomposed peat, s. g. 1.58	13	680	165	164
	14	669	174	173
	15	676	142	141
	16	680	79	74
	17	680	101	101
	18	679	131	127

TABLE 7

Comparison of the water content of a peat soil calculated according to formula (C) *with that found by drying the sample in a vacuum at 50°C.*

NUMBER OF THE SAMPLE	WATER CONTENT, ACCORDING TO (*C*)	WATER CONTENT, DRIED AT 50°C. IN VACUUM
	per cent	*per cent*
7	72.7	72.5
8	72.6	72.8
9	75.6	75.0
10	75.5	74.3
11	78.3	77.8
12	79.7	78.1

When this is considered, the difference between columns 3–4 and 7–8 will be within the limits of error.

Similar investigations were made on a heavy clay soil, the texture of which was as follows:

	per cent
Coarse sand (2–0.2 mm.)	5.3
Fine sand + coarse silt (0.2–0.02 mm.)	12.3
Fine silt (0.02–0.002 mm.)	18.4
Clay (<0.002 mm.)	51.5
Organic substance (loss on ignition with reduction)	10.1
Lime	2.4

The samples used were taken preliminary to a cultivation trial, where the porosity will be frequently checked with the porosimeter. The specific gravity was determined in the pyknometer and is, for an average of 12 determinations, 2.706 ± 0.008, on which value the figures in table 4 are based.

TABLE 8

Comparison of v_1 values determined by increased pressure with those obtained by decreased pressure

INCREASED PRESSURE AFTER ATMOSPHERIC PRESSURE	DECREASED PRESSURE AFTER INCREASED PRESSURE	DECREASED PRESSURE AFTER DECREASED PRESSURE	INCREASED PRESSURE AFTER DECREASED PRESSURE
100	104.8	104.8	100.0
100	99.1	99.1	99.1
100	101.9	101.0	98.1
100	105.1	104.1	100.0
100	103.1	104.2	100.0
100	102.9	102.9	99.6
100	103.6	101.8	99.1
100	103.6	103.6	100.0
100	106.3	106.3	99.0
100	103.1	103.1	100.0
100	107.6	107.6	98.7
100	106.6	106.6	98.7
100	110.9	110.9	100.0
100	106.0	107.5	97.0
100	107.5	101.5	100.0
100	102.4	103.5	98.8
100	105.5	104.1	100.0
100	107.9	106.6	98.7

If sample 5 is disregarded, the calculated and the directly measured porosities are in almost absolute agreement.

In table 5 the water content, obtained by using the specific gravity figure of 2.706 ± 0.008 in formula (C), is compared with that found by drying the sample at 110°C. The agreement between the figures leaves nothing to be desired.

It is tiresome to determine the specific gravity necessary for calculating the figures in column 2, and we find it more convenient to dry a number of samples, after determination in the Porosimeter, and use the value for water content so found in formula (C), by which s may be solved. For hygroscopic soils, this procedure is undoubtedly preferable to working with quite dry soils in the

porosimeter. This was done on the 17 soil samples in table 5, and the specific gravity obtained was 2.716 ± 0.007. The difference between this value and that found in the pyknometer is thus 0.010 ± 0.011, i.e., no difference.

In order to prove the applicability of the porosimeter to organic soils, similar investigations were made on three unequally decomposed peaty soils. The calculated values in table 6 are based upon the specific gravity found in the pyknometer. The agreement between the two methods is remarkably good.

At present, we cannot adequately discuss the "degree of dryness" most suitable as a basis for determining the specific gravity of peaty soils. The

TABLE 9

Determination of air porosity in a heavy clay, under bare fallow, cultivated in two different ways

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	TOTAL AIR SPACE	AIR SPACE IN PER CENT OF WET SOIL
	cc.	*cc.*	
Fallow I—soil compact			
1	688	131	19.0
2	699	76	10.0
3	688	62	9.0
4	689	62	9.0
5	696	48	6.9
6	684	91	13.3
			Av. 11.4 ± 4.3
Fallow II—soil loose			
7	684	243	35.5
8	688	199	28.9
9	695	233	33.5
10	692	198	28.6
11	688	168	24.4
12	690	171	24.8
			Av. 29.3 ± 4.5

values here used, however, refer to peat substances dried to constant weight in a vacuum (10 mm. Hg) at 50°C.

To complete the figures for peat, the water content calculated by formula (*C*) and that found by drying one of the peat soils have been brought together in table 7. The specific gravity used was determined in the pyknometer.

When using the porosimeter it is possible to work with either an increased pressure or a decreased pressure.[1] To ascertain whether we will obtain the

[1] In this case, the air porosity is calculated in the following way:

$$v_a = \frac{f(b - t)}{t}$$

same result in both cases we have determined v_i partly by increased pressure and partly by decreased pressure, as well as after increased and decreased pressure. The results of these investigations are made clear in table 8. The value of v_i which was obtained after atmospheric pressure was set at 100. The change of the air volume in all cases was 45 cc.

At a glance, the differences between the figures obtained by working with increased pressure and those found by using decreased pressure, appear to be great. This is mainly explained by the different effects of the increased and decreased pressure on the rubber packing. For particular investigations, where more than relative precision is required, the average value from one determination by increased and one by decreased pressure may be used to advantage.

As an example of the practical value of the porosimeter, some figures from a field investigation are given in table 9. The soil was a heavy clay, under bare fallow, cultivated in two different ways. Under one, the soil was closely compact, and under the other, relatively loose. After heavy rain, the water did not penetrate into the soil receiving the first type of cultivation but penetrated freely into the second.

The samples were taken at not less than 10-m. intervals and to a depth of 10 cm. The difference between the samples, therefore, is relatively large, but the differences between the figures from fallows II and I are 17.9 ± 2.8, leaving no doubt as to the structural differences after the two types of cultivation.

SUMMARY

This paper describes a new method for the direct measurement of air space in soils based on Boyle's law. The apparatus, the porosimeter, also permits calculation, in a simple way, of the specific gravity, water content, and soil substance.

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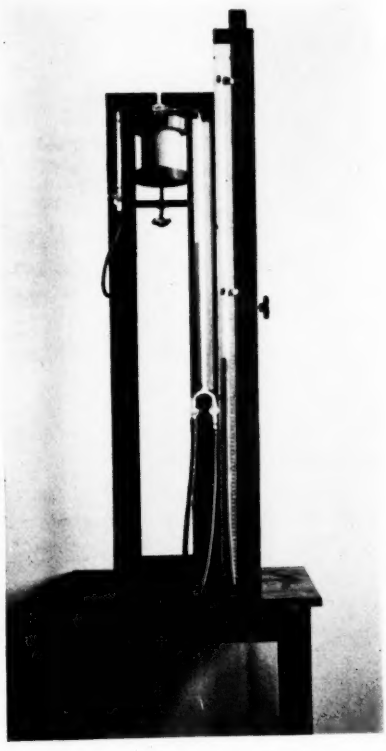
PLATE 1

THE POROSIMETER

PLATE 1



AVAILABLE CALCIUM A FACTOR IN SALT BALANCE FOR VEGETABLE CROPS[1]

VICTOR A. TIEDJENS AND L. G. SCHERMERHORN[2]

New Jersey Agricultural Experiment Station

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The availability of calcium in a soil has a tremendous effect on the quality of growth of vegetable crops. The type of fertilizer materials which are used for the growth of vegetable crops and the manner in which the soil should be handled depend on the potential calcium supply. Vegetable crops differ widely, and even varieties differ in their nutrient requirement with respect to available calcium. Fertilizer residues have a marked effect on exchangeable and available calcium as well as on the reaction of light cultivated soils. Observations made on vegetable farms in New Jersey, located on the sandy soils, show that pH is not always a reliable indication of the available calcium and that many of these sandy soils which have pH values[3] of 6.0 to 6.6, where large applications of soda and potash have been made, may be very deficient in calcium and magnesium. For acid soils or soils that have not received heavy applications of inorganic fertilizers the reaction test is a fairly reliable index of lime requirement. Cultivated soils on which liberal quantities of chemical fertilizers have been used, however, require a specific calcium test which should be interpreted on the basis of soil type and exchange capacity to indicate reliably the need for calcium.

Crops grown on soils having a high pH but a low available calcium supply respond differently to available soil moisture from those grown on soils having a more favorable nutrient balance. Observations on crops during several years of dry weather showed that the degree of injury varied with different farms even though soils were very similar in type. Truck crops on dairy farms where manure was used showed much less injury during dry weather than did those on farms where certain chemical fertilizers were used. Of the growers using all inorganic fertilizers some fared better than others. Those growers

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of vegetable gardening.

[2] It is a pleasure to acknowledge indebtedness to Drs. O. W. Davidson and L. Burkhart for suggestions and criticism on the manuscript; to Dr. Wm. Pierre, of the West Virginia Agricultural Experiment Station, and to Dr. E. E. Barnes, of the Ohio Agricultural Experiment Station, for suggestions and assistance; and to Mr. C. S. Cathcart for the Kjeldahl determinations.

[3] All pH values quoted in this paper were determined either by the hydrogen electrode or by the Hellige Klett colorimeter.

who were following a fertilizer program of lime and acid-forming fertilizers were growing better crops during dry weather than were those who used acid fertilizers without lime or those who used a neutral fertilizer in which neutrality was brought about by other means than lime. It was common to find a luxuriant growth of vegetable crops on some farms as long as moisture was fairly well distributed, but to find those same crops very badly damaged during short periods of dry weather. In many cases there were differences in color of foliage and type of growth which could not be associated with any nutrient deficiencies. Similar symptoms were noticed on greenhouse tomatoes and cucumbers where an attempt was made to control the moisture supply. Greenhouse growers have to contend with a wide range of weather conditions because they grow crops the year around. The type of growth of plants grown in the greenhouse can be regulated to a great extent by controlling the water supply. Crops growing on dry soil very quickly indicate whether the soil solution is in good condition for optimum growth because, if the nutrients are not properly balanced, deficiency symptoms for certain nutrients may be encountered even though there is an abundant potential supply of a particular nutrient present. In view of the fact that available calcium has played such a large part in correcting greenhouse soil problems a number of preliminary experiments were conducted to study the relation of available calcium to type of growth principally of cucumbers, tomatoes, and lima beans, and a few other crops such as onions, celery, and carrots.

LOW CALCIUM SOILS AND THE RESPONSE OF PLANTS TO THE ADDITION OF CALCIUM SALTS

Soils from farms on which problems existed, but where the reaction of the soil seemed satisfactory, were brought to the greenhouse, placed in pots, and treated with various chemicals. Seeds or plants of certain crops were then planted in them. In practically all such cases, the soils were brought to the greenhouse because crops did not respond to side-dressings of ammonium sulfate or sodium nitrate; or if there was a response, the type of growth was unsatisfactory and the harvested crop was of poor quality. Poor germination of lima beans; crooked, poorly filled string beans; brown roots on most crops; rough rooted carrots; burned heart leaves of celery; light green spots on celery leaves; onions that did not keep well when harvested; and tomato fruits that did not ship well because they were too soft, were common symptoms. Usually when the marketable crop was of poor quality, the foliage also had certain peculiar symptoms which were not particularly characteristic of those caused by extreme deficiencies.

These soils were tested by means of the quick soil tests, as recommended by Hester (2), and were supplemented in some cases with microchemical tests on the plant tissue. These tests showed a low amount of available calcium (less than 100 p.p.m.—a few cases indicated a negative test) but the usual moderate amounts of all of the fertilizer nutrients present in a mixed fertilizer.

Very often high concentrations of potassium were found, or if potassium was not high, it was assumed from the growers' practice and a high pH reading that sodium probably was present in liberal amounts even though the concentration was not determined.

The technique in these soil pot cultures was to supply sodium nitrate, calcium nitrate, calcium chloride, calcium carbonate (limestone), superphosphate, gypsum, and in some cases potassium nitrate, dolomite, and calcium-magnesium silicate slag to separate pots as a preliminary trial. Growth of the plants was then observed. Usually the results of the preliminary trial were sufficiently convincing to warrant the making of recommendations. Where the problem involved a calcium deficiency, the more soluble calcium salts gave a better response than those having a lower solubility. Calcium nitrate was highly effective. If the pots were kept well watered, superphosphate proved very beneficial, and gypsum was satisfactory. If the soil was kept quite dry, calcium nitrate gave a very good correction, but calcium sulfate was ineffective. Superphosphate was effective but not so effective as calcium nitrate. One difficulty with calcium nitrate was the necessity of supplying it at 3-week to monthly intervals to obtain best results. Dolomitic limestone usually gave very good results, and the correction was usually better than with calcium carbonate.

On these calcium-deficient soils sodium and potassium nitrate had depressing effects on growth, the degree of injury (unless some form of calcium was supplied with it) depended on whether the soil had abundant potassium or whether in the past it had been supplied with comparatively large amounts of sodium nitrate.

LOW CALCIUM-HIGH POTASSIUM SOILS AND PLANT GROWTH

One of the experiments on plant growth involving a greenhouse soil containing low calcium and high potassium is given as an example.

Cucumbers when freely watered in the greenhouse made a rapid growth in certain sections of the beds but were not normal. The internodes were long, probably as a result of the large amounts of water needed to grow the crop. The foliage was light green, and the leaves were small. They were grown on strings up to wires 7 feet above the soil. When the growing tip reached the wire, many of the veins of the older leaves showed a brown, water-soaked condition, which was soon followed by dying and gradual drying of the whole leaf, with the result that the plants soon had only the tip leaves left. Mosaic-like symptoms were present and were associated with malformed growing tips and leaves. After it was decided that mosaic was not the cause[4] the plants were examined microchemically. The tissue in question was packed with potassium and nitrate, but calcium was entirely lacking. The roots were kinky and rough, and the cortex had sloughed off prematurely.

[4] Dr. S. P. Doolittle of the U. S. Department of Agriculture examined these plants and said there was no mosaic present, but that the disturbance resembled a nutritional one.

Tomatoes growing in another house were tested, and similar observations were made. The tomatoes were growing much better and were fruiting but were not making a desirable growth; it was apparent that they were being affected by a similar nutritional disorder. The foliage was dotted with light green spots.

A quick chemical test (2) showed a pH range of 5.8 to 6.6.[5] Less than 50 p.p.m. of calcium was present. Potassium was exceptionally high and fluctuated with the water supply. As soon as a test could be made after a heavy application of water, the potassium was found to be very low but just before water was again applied, when the soil was quite dry, the potassium concentration was found to be very high. Apparently very little of the potassium was in the colloid and was easily leached to lower levels. The phosphorus concentration was approximately 70 p.p.m. Only a trace of magnesium was found, but the nitrate concentration was high. The soil baked very hard when dry and was very slippery when wet. It was classed as a medium sandy loam of a grayish black appearance although organic matter was comparatively low. A hardpan from one-half to 2 inches thick had formed under the plow depth, although drainage was satisfactory in most cases. This soil had been in use for 16 years.

The result of these observations indicated that a deficiency of calcium or magnesium or both was causing the symptoms. The roots resembled those of plants growing with insufficient calcium. The symptoms exhibited by the tops, however, did not resemble those of calcium or magnesium deficiency insofar as they were known. A macrochemical test of the soil was made. The soil colloid was found to be approximately 87 per cent calcium and 2 per cent magnesium saturated. The calcium content in the $6\frac{2}{3}$-inch acre depth was estimated to be equivalent to 7 tons of ground limestone. The potassium content was equivalent to 3.4 tons of muriate of potash.

Soil was taken from the poorest section of the beds, placed in 8-inch pots, and treated with individual salts. A cucumber plant was set in each pot. The amount of material applied, consisting of individual salts in each case, was equivalent to what would be found in a ton of 5–8–7 fertilizer or a ton of hydrated dolomite. The pots were watered just enough to prevent wilting.

Data from the pot cultures showed that soluble calcium salts gave a normal type of growth when compared with that of the injured plants grown in soil with no treatment. Calcium nitrate and calcium chloride were more effective than calcium sulfate. Plants grown with additional superphosphate, indicated some correction. It was later noticeable that calcium sulfate and superphosphate were more effective when the soil was kept more freely watered.

[5] The water used in this range came from wells and contained considerable sodium chloride and some calcium chloride. Records showed a pH reaction several years back of 7.6 to 8.4. The water was later taken from a brook, and a fertilizer mixture of ammonium sulfate, potassium sulfate, and superphosphate was used to lower the pH. This treatment had lowered the pH when the soil was examined for this experiment.

Plants grown with sodium or potassium salts gave no response or were injured when the sulfate ion served as the carrier. This injury was very characteristic of that found in the greenhouse.

Roots on plants supplied with lime were very extensive and vigorous and, instead of growing toward the outside of the pot and between the walls of the pot and soil, as was the case where more soluble salts were applied, they were distributed all through the soil to the extent that the soil crumbled to pieces instead of coming out in a solid ball when the pots were dumped. This indicated a difference in aeration of the soil.

From these results it was apparent that the correction was brought about by the addition or greater availability of calcium, as is shown by the addition of potassium sulfate or chloride. The sulfate tended to decrease calcium availability, thereby producing a low calcium-high potassium condition to the extent that the potassium prevented calcium from being absorbed by the plants, as has been suggested by Lundgårdh (3). Apparently calcium sulfate was not sufficiently soluble in soils with low moisture content. The beneficial effect of calcium nitrate endured for 3 to 4 weeks, and repeated applications had to be made.

The relationship between calcium and potassium was repeated in sand culture. It was found that as long as calcium was maintained at a high level (0.0090 p.v.m. conc. of 1 atmos. solution) it was impossible to injure the growth. If, however, the calcium concentration was maintained at a low level (0.0014 p.v.m. conc.) the effect of large amounts of potassium was to produce a type of growth that was not symptomatic of any particular deficiency but was soft and succulent, as though the plants were watery in texture, and the foliage had a yellowish green cast resembling plants that do not assimilate nitrate readily. A discussion of this whole problem, interpreting the relation of calcium to potassium has been given by Lundegårdh (3). As far as the effect of the relationship of calcium to potassium is concerned, these results agree with his observations.

As a result of these observations, a recommendation was made that 1 ton of dolomitic limestone be used per acre each year, unless the soil reaction test indicated otherwise, that calcium nitrate be used as the source of nitrogen and superphosphate as the source of phosphoric acid, and that potash be withheld until a test showed that it was needed. Spent mushroom manure, which added some potassium, was being used on the beds.

Two years after the treatment was recommended, the soil was examined. Crop growth was much improved, and the roots on both cucumbers and tomatoes were white and vigorous. A test showed the presence of sufficient available calcium and of potash in liberal quantities. The soil no longer baked when dry nor did it pack down so firmly as before. In the past, nematodes had been a problem and steam sterilization of the soil did not seem to eradicate the pest completely because it was difficult to get the hard clumps of soil heated through. At the present time, the soil heats through readily and thorough

eradication is effected with much less heating. The excessive heating and the poor aeration of the soil in the past undoubtedly contributed to the poor growth, but apparently the correction of this soil depended on one of the many functions of calcium directly in the soil and indirectly on the plant growth.

The similarity of growth of vegetable crops on the coastal plain soils of New Jersey to the growth of plants observed in greenhouse soils prompted further experiments in sand and soil pot cultures to determine, if possible, how the chemical composition of the plant was changed by different ratios of available calcium to other cations. Some results on sodium are presented at this time.

TABLE 1

Partial volume molecular concentration of nutrient solutions used in determining the relation of calcium-sodium ratio to the growth of the tomato

SOLUTION*	KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	$NaNO_3$†	$CaCl_2$	NaCl
A, 3 atmos.	0.0045	0.0045	0.0405	0.0045	.....	.....
B, 3 atmos.	0.0045	0.0045	0.0225	0.0225	.....	.....
C, 3 atmos.	0.0045	0.0045	0.0045	0.0405	.....	.....
D, 1.75 atmos.	0.0045	0.0045	0.0203	0.0023	.....	.....
E, 1.75 atmos.	0.0045	0.0045	0.0113	0.0113	.....	.....
F, 1.75 atmos.	0.0045	0.0045	0.0068	0.0156	.....	.....
G, 1.75 atmos.	0.0045	0.0045	0.0023	0.0203	.....	.....
H, 1.5 atmos.	0.00675	0.00235	0.02025	0.00235	.....	.....
I, 1.5 atmos.	0.00675	0.00235	0.00235	0.02025	.....	.....
J, 1.5 atmos.	0.00675	0.00235	0.00235	.....	0.02025	.....
K, 1.5 atmos.	0.00675	0.00235	0.00235	.....	.....	0.02025

* Concentrations are approximate and varied somewhat from values stated.

† The sodium nitrate furnished one-half as much nitrate as the calcium nitrate. This arrangement seemed necessary to keep calcium and sodium more comparable for purposes of this investigation. The same is true of the chloride content.

CALCIUM AND SODIUM RATIO AND GROWTH OF TOMATO IN SAND CULTURE

Studies on the effect of calcium and sodium ratio on plant growth were made on the tomato because of the various peculiar types of growth found in fields of canhouse tomatoes. A critical examination of the plants and of the soil showed that the problem was not one involving potassium because it was not being used in sufficiently large amounts to approach the concentrations which would produce abnormal growth of the foliage. Since growers, however, were using large amounts of sodium nitrate and very little calcium-carrying material, it seemed that a study on the effect of varying the calcium to sodium ratio on the growth of the tomato would be helpful as a guide to diagnosing peculiar growth conditions in the field even though the extreme ratios used in the pot cultures might not necessarily be encountered under field conditions.

Tomato plants were set in 3-gallon coffee urns in white, sifted, washed pit sand, and nutrients were applied by a continuous drip system. Eighteen-liter

inverted bottles served as reservoirs to supply the nutrients to five crocks, by means of asphalt-coated ¾-inch pipe and small pieces of capillary tubing at each crock to regulate the flow so that 3 liters were supplied each crock during 20 hours.

The nutrient solutions used are given in table 1. Two lots of plants were grown.

Plants in lot 1 were grown from February 5 to March 5 with solutions A, B, and C. Solutions having an osmotic concentration of approximately 3 atmospheres pressure were used because, during this time of the year when much cloudy weather prevails, plants grown in a more concentrated nutrient salt medium produce a less succulent growth which is more comparable to that of plants grown with abundant sunshine. The initial plants placed in the crocks were 6 to 7 inches high, were high in carbohydrates and low in nitrates, and had

TABLE 2

Dry matter in organs of tomato plants grown with nutrient solutions containing different ratios of calcium to sodium

SOLUTION	STEMS		PETIOLES		BLADES	
	Lower	Upper	Lower	Upper	Lower leaves	Upper leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Iintial plants*	20.6	....	11.9	....	19.1	....
A, 9 Ca–1 Na	17.5	11.7	11.3	10.5	14.2	14.9
B, 5 Ca–5 Na	16.3	10.8	10.6	10.1	12.6	14.5
C, 1 Ca–9 Na	16.2	9.4	9.5	8.6	10.7	13.3
D, 9 Ca–1 Na	7.1		7.0		12.7	
E, 5 Ca–5 Na	6.5		6.3		11.2	
F, 3 Ca–7 Na	6.1		5.8		10.1	
G, 1 Ca–9 Na	6.0		5.4		9.3	

* Plants supplied with solutions A, B, and C were grown from February 5 to March 5. Plants grown with solution D, E, F, and G were grown from March 6 to April 3.

a gray-green appearance. A composite sample for dry matter determinations was made on blades, petioles, and stems. When the plants in the crocks were harvested, the upper and lower blades, petioles, and stems were analyzed separately.

Plants in lot 2 were grown with solutions D, E, F, and G. They were 2 inches tall, were comparatively soft and succulent, were dark green in color, and contained appreciable amounts of nitrates, but little starch. They were set in the crocks on March 6 and harvested on April 3. The solutions used had an approximate concentration of 1.75 atmospheres, inasmuch as light conditions were more favorable than for the group 1 plants.

The data on percentage of dry matter for both lots are given in table 2, carbohydrate fractions in table 4, and nitrogenous fractions for plants in lot 2 in table 3.

Regardless of the part of the plant examined, the data from plants grown with solutions A, B, C, (table 2) show a decrease in dry matter as the proportion of calcium is decreased and sodium is increased from a ratio of 9:1 to 1:9.[6] For example, the old stems decreased 1.3 per cent, the old petioles 1.8 per cent, and the old leaf blades 3.5 per cent, whereas the upper stems (younger tissue) decreased 2.3 per cent, the upper petioles 1.9 per cent, and the younger leaf blades 1.6 per cent. The younger plants, which were more succulent, grown

TABLE 3

Nitrogenous fractions of different parts of tomato plants grown with solutions in which the ratio of calcium to sodium was varied

Data expressed as percentage of green and dry matter

SOLUTION	TOTAL ORGANIC N		PROTEIN N		SOLUBLE ORGANIC N		NITRATE AND AMMONIUM N	
	Green	Dry	Green	Dry	Green	Dry	Green	Dry
Tomato stems								
D, 9 Ca–1 Na	0.1401	1.90	0.0801	1.05	0.0600	0.85	0.0800	1.12
E, 5 Ca–5 Na	0.1041	1.60	0.0757	1.16	0.0284	0.44	0.0876	1.35
F, 3 Ca–7 Na	0.0974	1.62	0.0670	1.12	0.0304	0.50	0.0856	1.40
G, 1 Ca–9 Na	0.0800	1.33	0.0552	0.92	0.0248	0.41	0.0856	1.43
Tomato leaf blades								
D, 9 Ca–1 Na	0.6230	4.84	0.5278	4.09	0.0952	0.75	0.0424	0.33
E, 5 Ca–5 Na	0.5277	4.67	0.4301	3.80	0.0976	0.87	0.0424	0.38
F, 3 Ca–7 Na	0.4789	4.75	0.3833	3.80	0.0956	0.95	0.0564	0.56
G, 1 Ca–9 Na	0.4229	4.55	0.3497	3.76	0.0732	0.79	0.0700	0.75
Tomato leaf petioles								
D	0.1248	1.76	0.1004	1.41	0.0244	0.35	0.1692	2.41
E	0.0984	1.52	0.0762	1.17	0.0222	0.35	0.1498	2.39
F	0.0926	1.49	0.0726	1.14	0.0200	0.35	0.1400	2.41
G	0.0630	1.17	0.0516	0.96	0.0114	0.21	0.1406	2.60

with solutions D, E, F, and G likewise showed similar trends, decreasing 1.1 per cent in the stems, 1.6 per cent in the petioles, and 3.4 per cent in the leaf blades.

[6] The ratios of calcium to sodium used in these experiments are extreme to facilitate interpretation of data for narrower ratios. It is doubtful whether these extremes would be encountered on coastal plain soils of New Jersey except in special temporary cases where the potential supply of calcium in the soil was low and a heavy application of nitrate of soda had been made. A number of cases have been identified in the culture of late celery where a heavy rain was preceded by a heavy application of sodium nitrate. Under such conditions leaching was sufficiently severe to carry the replaced calcium out of reach of the feeding roots. Complete calcium deficiency symptoms have been observed under such conditions.

In the plants grown in the low calcium-high sodium treatment[7] there was a consistent decrease in assimilated organic nitrogen (table 3) while the mineral nitrogen (nitrate and ammonium) showed no significant difference except in the leaf blades where the nitrate content increased while protein nitrogen decreased.

The carbohydrates decreased (table 4), although not so consistently as did the organic nitrogen, as the ratio of calcium to sodium changed from 9:1 to 1:9. The largest difference was between the 9:1 and the 5:5 ratios. The weight of the plants in the various groups was very similar. The high calcium

TABLE 4

Carbohydrate fractions in the blades and stems of tomato plants grown with nutrient solutions in which different proportions of calcium to sodium ions were present

Data expressed as percentage of green matter

SOLUTION	REDUCING SUGAR		TOTAL SUGAR		STARCH		HEMICELLULOSE	
	Blades	Items	Blades	Stems	Blades	Stems	Blades	Stems
Initial plants	1.008	1.778	1.104	2.221	6.156	3.121	0.287	0.226
Upper blades and stems								
A, 9 Ca–1 Na	0.675	0.679	1.062	1.203	0.700	0.220	0.206	0.296
B, 5 Ca–5 Na	0.674	0.524	0.841	0.967	0.384	0.178	0.186	0.216
C, 1 Ca–9 Na	0.359	0.531	0.804	0.919	0.297	0.160	0.219	0.244
Lower blades and stems								
A	0.395	0.670	0.618	1.642	0.366	0.670	0.182	0.294
B	0.390	0.579	0.567	1.549	0.199	0.693	0.130	0.290
C	0.233	0.612	0.399	1.575	0.171	0.483	0.139	0.421
Total blades and stems								
D, 9 Ca–1 Na	0.340	0.265	0.692	0.414	0.296	0.085	0.152	0.226
E, 5 Ca–5 Na	0.196	0.171	0.491	0.343	0.146	0.093	0.146	0.190
F, 3 Ca–7 Na	0.076	0.177	0.136	0.262	0.064	0.054	0.089	0.154
G, 1 Ca–9 Na	0.086	0.183	0.140	0.260	0.140	0.063	0.049	0.141

plants were more uniformly dark green in color than the high sodium plants. The general appearance of the plants indicated a difference in concentration of soluble organic nitrogen. Even though the high and low calcium soultions contained different amounts of nitrate (made necessary by the substitution of monovalent sodium for divalent calcium) the concentration of nitrate in the plants was similar (table 3).

[7] The methods were the same as those used for chemical analysis of tissue described in the New Jersey Agricultural Experiment Station Bulletin 547, but modified to include the method of extraction suggested by Davidson et al. in *Plant Physiology* 9: 817–822.

CALCIUM: SODIUM RATIO AND GROWTH OF TOMATO WITH HIGH AND LOW NITRATE SUPPLY

Two lots of plants, each consisting of 60 crocks with 3 tomatoes in each, were grown in sand culture, one with solution D and the other with solution G (table 1). The plan was to shift these to other solutions when the plants were 12 to 15 inches high. Before the plants could be shifted, however, a mosaic disease made its appearance and killed the plants in 55 of the crocks supplied with solution G (high sodium) but did only slight damage to the plants grown in solution D (low sodium). Although this was not part of the experiment, it was a good indication that these experiments were producing a type of growth which was similar to, and fully as susceptible to weather conditions and disease as, that of many tomato plants growing in the fields which were low in available calcium. The watery, light green, succulent growth of the high sodium plants grown with solution G and the darker green, less succulent plants grown with the high calcium solution D, even though of the same size, were characteristic of types of growth associated with good and poor growing conditions in the field.

The experiment was repeated. Although the tomato plants in the two groups were very similar to the plants in the two previous groups, they grew free of mosaic. Two 4-inch seedling plants comparatively soft and succulent and dark green were placed in each of the crocks on January 20, one-half being supplied with solution H and the other half with solution I (table 1), until March 1 when they were 12 to 14 inches high. At this time the plants receiving solution H (high calcium) were dark green, succulent, and growing rapidly. The other plants grown with solution I (high sodium nitrate) were slightly larger but lighter green and seemed to be more succulent than the calcium group. They wilted more easily when the day temperature increased as a result of bright sunshine. On March 2 the 120 plants grown with solution H were divided into four groups. Group 1 was continued with the same solution; group 2 was given solution I (high sodium nitrate); group 3 was given solution J (high calcium chloride in place of calcium nitrate); and group 4 was given solution K (high sodium chloride).

The 120 plants grown with solution I (high sodium nitrate) were likewise divided and treated as the previous group.

The plants were harvested on March 11, 9 days after being shifted to different solutions. The short interval elapsing between the shifting of solutions and harvesting was necessary to prevent excessive hardening due to accumulation of carbohydrates in the low nitrate groups. This accumulation of carbohydrates overshadowed the assimilation of the nitrate ion and consequently tended to mask the cation effect. Only the stems were harvested and analyzed. They were divided into 10 inches of base and 6 to 10 inches of tip of stem tissue. The dry matter and the nitrogenous fractions are shown in table 5.

There was very little difference in percentage of dry matter in the base of

the stems. In the upper portion of the stems, the results agree with those in table 2 in that there was 9.8 per cent dry matter in plants grown with sodium nitrate as compared with 11.0 per cent in those grown with calcium nitrate. The plants in the latter treatment, however, did not decrease in dry matter when shifted to sodium nitrate, as was expected; but the sodium nitrate plants did increase in dry matter when shifted to calcium nitrate, as was expected. When either group was shifted to the low nitrate solutions, carbohydrate accumulation apparently increased the dry matter still further. This increase was accentuated more by sodium chloride than by calcium chloride, apparently because nitrate assimilation is at a lower level where sodium is increased. This permits carbohydrates to accumulate more rapidly than where calcium is maintained at a higher level.

TABLE 5

Dry matter and nitrogenous fractions in the lower and upper stems of tomato plants after being shifted from one nutrient solution to another

SOLUTION* CHANGE	DRY MATTER		NITRATE N		SOLUBLE ORGANIC		PROTEIN N		TOTAL ORGANIC N	
	Lower stem	Upper stem	Lower stem	Upper stem	Lower stem	Upper stem	Lower stem	Upper stem	Lower stem	Upper stem
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H to H	7.0	11.0	0.0817	0.0866	0.0829	0.1034	0.0828	0.1147	0.1757	0.2181
H to I	7.0	11.5	0.0821	0.0807	0.0503	0.0901	0.0888	0.1065	0.1391	0.1966
H to J	7.2	12.1	0.0369	0.0389	0.0475	0.0778	0.0905	0.0976	0.1380	0.1754
H to K	7.2	12.3	0.0457	0.0331	0.0563	0.0917	0.0743	0.1027	0.1306	0.1944
I to I	7.0	9.8	0.0739	0.0807	0.0553	0.0653	0.0805	0.0987	0.1358	0.1640
I to H	7.0	11.0	0.0866	0.0816	0.0734	0.1028	0.1081	0.1236	0.1815	0.2260
I to J	7.0	11.1	0.0428	0.0273	0.0502	0.0847	0.0750	0.1035	0.1252	0.1882
I to K	7.0	13.0	0.0321	0.0369	0.0657	0.0719	0.0814	0.1057	0.1471	0.1776

* Composition of nutrient solutions given in table 1 (H = high calcium nitrate, I = high sodium nitrate, J = high calcium chloride, K = high sodium chloride).

The data on nitrogenous fractions are in better agreement with those given in table 2 than are dry matter determinations. Soluble organic nitrogen was much higher in the calcium nitrate plants than in the sodium nitrate plants. This is shown by the comparison between groups H and I. Where H was shifted to I, amino acids decreased, but where the shift was made from solution I to H amino acids increased. This holds as long as there was sufficient nitrate nitrogen present to prevent carbohydrate accumulation. The difference in amino acids was greater when the plants were shifted from solution I to H than from solution H to I. Had the plants been grown for a longer period on the shifted solutions, it is probable that even the H to I (Ca to Na) shift would have shown a greater difference. The protein nitrogen shows similar trends, although the differences were not so great as in the more quickly assimilated amino acids.

DISCUSSION

The importance of calcium in the nutrition of plants has been shown by Prianischnikov (6) and by Nightingale et al. (5). The importance of the calcium ion as an antagonizer of all other cations necessary for plant growth has likewise been shown (7). The data and observations presented in tables 2 to 5

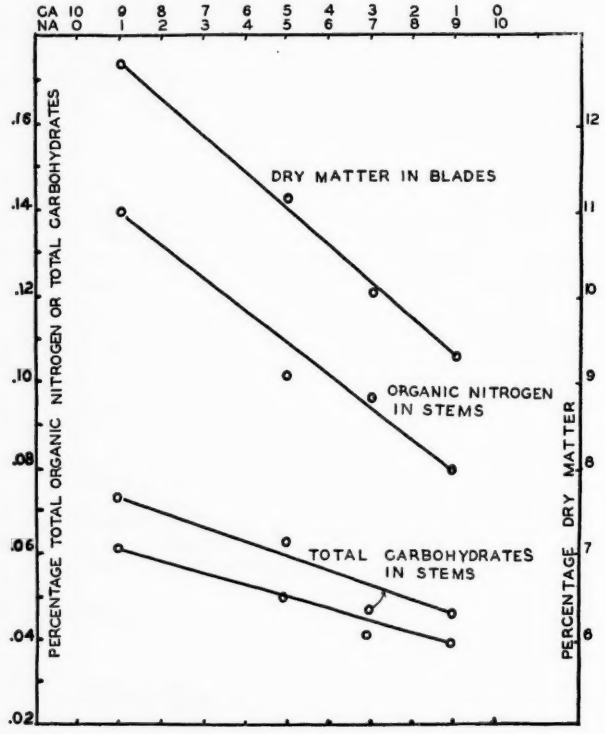

FIG. 1. EFFECT OF DIFFERENT RATIOS OF CALCIUM TO SODIUM ON DRY MATTER, ORGANIC NITROGEN, AND CARBOHYDRATE FRACTIONS IN TOMATO

inclusive further emphasize the importance of the calcium ion in regulating the growth of the plant.

Plants grown with an optimum supply of calcium apparently are dark green, possess a composition that is resistant to changes in environment, and present few physiological disorders because they assimilate nitrogen and carbon most rapidly. Even though the plants in the two groups, one grown with an optimum calcium supply and the other grown with a similar cation supply but comparatively low in calcium, made an equal volume of growth, but the quality

of growth was vastly different. As the calcium content in the nutrient solution decreased and the sodium increased, the tissue contained more water. Under conditions of rapid transpiration (lowered relative humidity) the high calcium plants showed no signs of wilting, whereas the low calcium plants wilted severely. In these cultures the ratio of calcium to sodium was not low enough to cause injury to the roots or to cause proteolysis as was suggested by Nightingale (5) in the case where calcium concentration was too low to support synthetic processes. In figure 1 are shown trends for dry matter, organic, nitrogenous, and carbohydrate fractions from plants grown with different amounts of calcium. A decrease in calcium is accompanied by a decrease in synthesized products. Were the curves extended to the point where there was no longer sufficient calcium to support synthetic processes, organic nitrogen would probably decrease but carbohydrates would increase rapidly for a short period until proteolysis disintegrated the carbohydrates more rapidly than they were being synthesized.

The difference in water content may be explained by the difference in affinity for water by calcium and sodium ions, as was pointed out by Flint (1). Mattson (4) has shown a difference in dispersion due to hydration of proteins when associated with calcium or sodium ions. It would seem possible that there are certain associations between sodium and proteins in the cell which would require the presence of more water. The dehydrating effect of the calcium ion introduced into such a system would tend to counteract or antagonize the effect of the sodium. In view of the fact that potassium has a similar effect to sodium, although to a smaller degree, it is entirely possible that one of the functions of potassium when absorbed is to counteract the dehydrating effect of an over-abundance of calcium.

From these results it would seem that a very important function of calcium is to hold the cell protoplasm in an equilibrium conducive to synthetic processes that build up proteins rapidly. This accumulation of proteins in turn gives stability to protoplasm, the amount of calcium needed for optimum synthesis being determined by the heredity of the plant. Thus plants which require a high pH likewise require a high calcium supply. A high soil pH brought about by ions other than calcium is not suitable because the physical properties when absorbed by the plant do not support synthetic processes as well. Thus the tomato which has a lower calcium requirement than the lima bean is less sensitive to soil conditions and tolerates a wider range of ratios than the lima bean, which makes very poor growth on soils that have a low calcium but otherwise high cation content. Many of our leguminous crops synthesize large amounts of proteins and also have a high calcium requirement.

A high proportion of protein to soluble organic nitrogen determines a certain quality (color and hardness) of growth. A high percentage of organic nitrogen means more dry matter and less water, or more substance to a given volume of growth. It is a common observation that spinach grown on a well-limed soil has a certain quality about it that prevents it from becoming yellow in

color and soft in texture after a heavy rain. The grower who does not use much lime complains of losing a crop of spinach because of a heavy rain. A comparison of spinach from a well-limed and a poorly limed field shows the difference in texture. Celery will wilt, turn yellow, and die following a heavy rain on soil low in calcium but freely supplied with other cations. The importance of the ratio of calcium to other cations, which probably is the cause of many of our nutritional problems on the coastal plain soils, cannot be over-estimated. The effect it has on the quality of growth makes it possible to blame much of our quality problems on the lack of available calcium (changing of ratio of ions) in the soil solution, particularly since its availability may vary between conditions of drought and abundant rainfall.

From the results presented in the discussion on the relation of the calcium to sodium and potassium ion it is safe to assume that even though sodium is not important as a nutrient for plant growth it probably has a marked effect on plant growth and is worthy of serious consideration in our fertilizer practices. It also offers a clue to the advantages and disadvantages of different nitrate carriers on different types or soils containing different proportions of cations.

SUMMARY

Soil and sand culture experiments are reported which show that soils may have a satisfactory reaction for crop growth but have too little available calcium for optimum synthesis of protein and carbohydrate materials.

An abundance of potassium may prevent the absorption of sufficient calcium on media low in calcium.

Plants grown with a ratio of 1 part calcium to 9 parts of sodium had from 1 to 3 per cent less dry matter than did plants grown with 9 parts of calcium and 1 part of sodium.

Associated with this decrease in dry matter, there was a poorer type of growth, which made plants more susceptible to wilting, to certain constitutional disorders, and to severe injury during periods of drought or heavy rainfall.

The ratio of available calcium to potassium or sodium was extremely important for germination of seed and growth of vegetable crops on coastal plain soils.

It is suggested that the effect of cations on growth is due to the hydration of protoplasm induced by different cations.

Nitrate-carrying salts apparently have other functions in the plant than merely supplying the necessary nitrogen for growth, a fact worthy of consideration in fertilizer practices.

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DEFICIENCY CHLOROSES IN CITRUS

A. R. C. HAAS

University of California Citrus Experiment Station

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It is most difficult in the field to understand the causes of some physiological diseases of citrus that manifest themselves as chloroses in the leaves. A lack of iron is usually considered the cause of chlorosis when, in fact, there are chloroses that result from any number of different causes. The present paper does not deal with iron chlorosis but rather points out the symptoms of chlorosis that result from other so-called deficiencies such as manganese, sulfate, and magnesium; it is recognized, however, that there are many more types of chlorosis, such as calcium chlorosis (6) and zinc chlorosis.

The factors in these chloroses usually are most involved, and every clue may become a valuable aid in the diagnosis. In some cases a basis may be obtained by comparison of field data with the growth of citrus in solution or sand cultures under controlled conditions.

Figure 1, plate 1, illustrates the excellent growth of citrus roots of budded Valencia orange trees when grown in glazed crocks of 12-gallon capacity and when supplied with the nutrient salts necessary for healthy growth.

With the proper technique, budded citrus trees also may be grown in distilled water to which is added certain chemically pure inorganic salts (3). Figure 2, plate 1, shows the growth of budded Valencia orange trees in such solution cultures in 10-gallon acid carboys (neck portion removed). Aeration of deep solutions is necessary for healthy growth over any extended period. These methods of growing citrus under artificially controlled conditions were used in the present study of manganese, sulfate, and magnesium chloroses.

MANGANESE CHLOROSIS

The effects of a deficiency of manganese in the growth of some varieties of citrus have already been described (3). By means of solution cultures with budded Valencia orange trees and the use of a manganese-free iron supply it was possible to produce trees such as shown in figure 2, plate 1, with leaves bearing symptoms of chlorosis as a result of manganese deficiency. It should be pointed out that chlorosis in citrus may also be brought about by an excess of manganese (4).

Figure 1*A* and *B*, plate 2, illustrates the chlorosis in Valencia orange leaves produced in solution cultures by means of a manganese deficiency. The chlorotic spots may be small as in *A* or large as in *B*. The same type of chlorotic

spotting (pl. 2, fig. 1*C*) frequently is seen in the leaves of trees grown in soil containing considerable calcium carbonate. Steinberg (8) has shown that calcium carbonate may be utilized in solutions as a means of removing the last traces of the heavy metals, including manganese.

SULFATE CHLOROSIS

There are no known instances of chlorosis due to a sulfate deficiency occurring in the citrus districts of California. In order to know the appearance of the chlorosis that results from a sulfate deficiency, budded Valencia orange trees were grown for several years in 12-gallon glazed earthenware crocks filled with pure silica sand. The culture solution employed (3) was the same as that used for the control cultures (the roots of one of which is shown in figure 1, plate 1) except that nitrate was substituted for sulfate.

Figure 2, plate 2, shows the chlorotic appearance of the leaves. The total sulfur content in the dry matter of the various portions of these trees was compared with that in the dry matter of corresponding portions of trees of the same age grown in an adjacent nursery. The results are given in table 1.

TABLE 1

Total sulfur as percentage of dry matter

PORTION OF TREE	TREES GROWN IN PURE SILICA SAND WITHOUT SULFATE	TREES GROWN IN SOIL CONTAINING SULFATE
New mature leaves	0.048	0.233
New mature twigs	0.014	0.029
Trunk scion bark	0.036	0.017
Trunk root bark	0.031	0.077
Young rootlets	0.066	0.079

The chlorotic leaves of sulfate-deficient trees in sand cultures contained about one-fifth the percentage of total sulfur found in leaves of healthy trees grown in soil. The results show that sulfate-deficiency in citrus brings about a chlorosis in the leaves.

MAGNESIUM CHLOROSIS

Magnesium deficiency and the chlorosis caused by it have received most consideration in connection with the growth of tobacco and other crops (1) on sandy soils of eastern United States. A chlorosis of citrus due to a magnesium deficiency recently was described by Parbery (5) but is not illustrated. This physiological disease occurs in New South Wales. Its description agrees very well with that of the symptoms artificially produced (7) by a magnesium deficiency in pure silica sand cultures. Figure 1, plate 3, illustrates the chlorosis in Valencia orange leaves when the trees were grown in pure silica sand with a

culture solution lacking magnesium (7). As the chlorosis advances the bronzing effect on the leaf color increases.

Recently, chlorotic leaves were found on trees in southern California in locations close to the irrigation line; it is apparent that the trees have been overwatered at times with the loss of soluble magnesium as a possibility. Figure 2, plate 3, illustrates the symptoms of chlorosis found in the leaves of these trees. They resemble very closely those produced (7) in sand cultures when magnesium was deficient.

Chlorotic leaves from the trees from which those shown in figure 2, plate 3, were obtained, were found to contain 0.08 per cent of magnesium and 5.42 per cent of calcium in the dry matter, whereas healthy (control) leaves from trees of nearby unaffected groves contained 0.68 per cent of magnesium and 4.35 per cent of calcium respectively. Leaves from other affected trees contained 0.06 per cent of magnesium and 5.42 per cent of calcium in the dry matter, whereas control leaves from healthy trees in the same grove as the affected trees contained 0.30 per cent of magnesium and 5.46 per cent of calcium respectively.

Citrus bronzing in Florida (2) is considered to be a chlorosis that results from a magnesium deficiency.

It should be evident from these results that a chlorosis may be brought about by any of several causes. Whether or not iron within the tree is in some way affected in any or all of these chloroses is at present unknown. A knowledge of differences in the types of chlorosis symptoms produced physiologically in citrus leaves by a deficiency or an excess of certain elements may be of assistance in the diagnosis and control of chlorosis diseases.

SUMMARY

A study was made of the chloroses brought about in citrus trees by a deficiency of manganese, sulfate, and magnesium. Sand, soil, and solution cultures were used as media in which the trees were grown.

Manganese deficiency in orange trees was accompanied by a chlorotic spotting of the leaves. Such symptoms have been noted frequently in the field under conditions of excessive calcium carbonate.

The chlorosis that results from a deficiency of sulfate requires considerable time for the symptoms to become manifest and consists of a general yellowing of the leaf with the veins remaining green until the chlorosis becomes extreme.

When a deficiency of magnesium affects citrus leaves, the chlorosis at first occurs as a yellow stripe on each side of the dark green midrib. Later the leaves assume a bronze color.

A deficiency of any of these three elements in citrus is accompanied by a chlorosis which is more or less characteristic for the deficient element. A knowledge of the symptoms corresponding to certain deficiencies is of help in the diagnosis of physiological diseases in the field.

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PLATE 1

FIG. 1. Root system of a Valencia orange tree grown in pure silica sand in a glazed earthenware crock of 12-gallon capacity. Distilled water containing chemically pure salts (3) was used to maintain healthy growth.

FIG. 2. Growth of a budded Valencia orange tree in an aerated solution culture (3) in which phosphate was present for only a few days prior to the renewal of the solution and in which 5 to 10 p.p.m. of aluminum was present when phosphate was absent. This is the largest citrus tree grown to date in a solution culture. The ruler placed next to the trunk is 1 foot long.

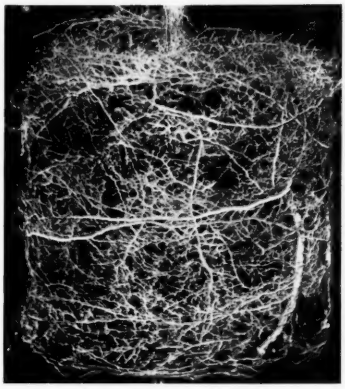

FIG. 1

FIG. 2

PLATE 2

FIG. 1. Chlorosis as a result of manganese deficiency in Valencia orange leaves. *A*, dorsal (upper) surface of leaf from tree grown in a controlled culture solution lacking manganese. *B*, translucent spots as seen from the ventral (lower) surface of a leaf obtained from a tree grown in a manganese-deficient culture solution. *C*, leaves from trees in the field where the soil contained large amounts of calcium carbonate.

FIG. 2. Chlorosis in Valencia orange leaves from trees grown in pure silica sand to which was applied a culture solution lacking sulfate.

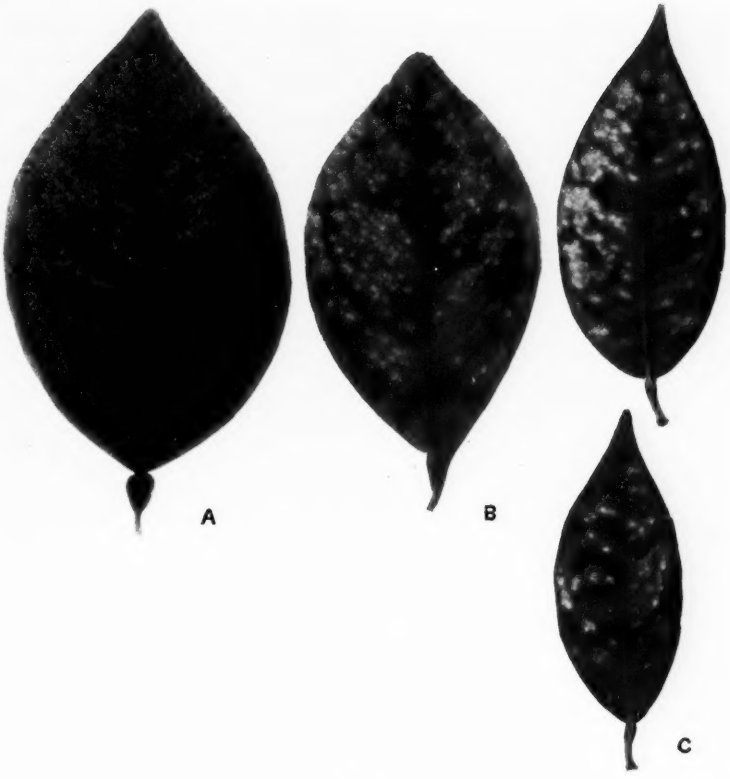

FIG. 1

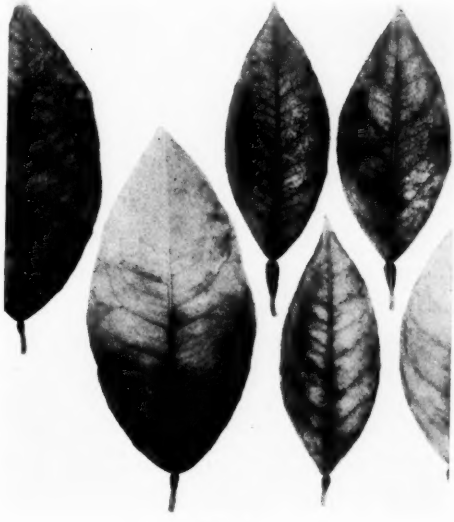

FIG. 2

PLATE 3

FIG. 1. Chlorosis of Valencia orange leaves obtained from trees grown in pure silica sand to which a culture solution lacking magnesium was applied. Note the increase in the bronzing effect as the chlorosis becomes more severe.

FIG. 2. Chlorosis of Valencia orange leaves obtained from trees in the field in locations close to the irrigation line. Overwatering may be responsible for the loss of magnesium and the production of magnesium-deficiency chlorosis.

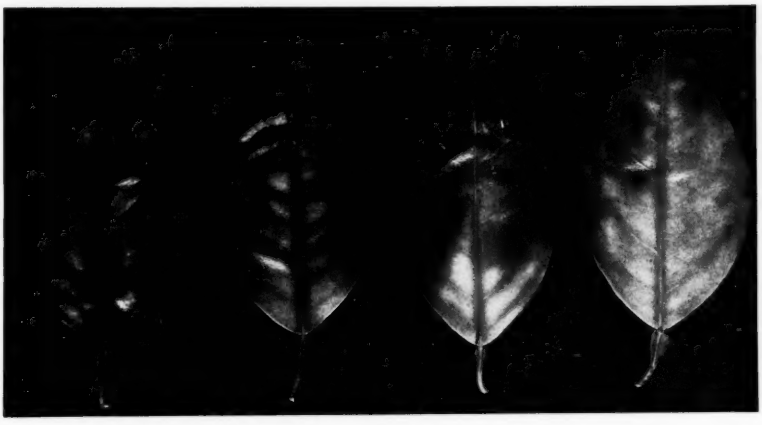

FIG. 1

FIG. 2

THE TOLERANCE OF NITRATE BY PURE CULTURES OF AZOTOBACTER[1]

P. L. GAINEY

Kansas Agricultural Experiment Station

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There are numerous references in the bacteriological literature to both a beneficial and an injurious effect of nitrates upon Azotobacter. A detailed discussion of these apparently conflicting data is not necessary in this connection. Suffice it to say that a general survey of the available information has led to the conclusion that in relatively low concentrations nitrates are beneficial, or, at least, not injurious. On the other hand, if the concentration is relatively high an injurious effect is produced, not, however, necessarily due to a directly toxic effect upon the Azotobacter cell but possibly, in soil at least, to an indirect effect upon some environmental factor.

In recent bacteriological and chemical studies of soil from "fertile" and "nonfertile"[2] areas taken in close proximity, "the density of the Azotobacter population"[3] was repeatedly observed to be greater in the less productive soil. At the same time the concentration of nitrates was almost invariably greater in the more productive soil. Fertile soils of the type studied produced, as an average for the 5 years during which yields were recorded, 2.4 times as much grain and 3.0 times as much protein as did the nonfertile soil. Elsewhere[4] data are being presented which show that this difference in productivity can be explained wholly on the basis of differences in available nitrogen. Inasmuch as this inverse relationship between the Azotobacter population and the nitrate content was so consistently observed, the inference might be drawn that the lower Azotobacter population resulted from the higher nitrate content, but the

[1] Contribution No. 170, department of bacteriology, Kansas Agricultural Experiment Station.

[2] The term "fertile" as here used has reference to the well-defined, darker green and more vigorously growing areas, usually 2 to 3 feet in diameter, described and pictured in "The rôle of nitrogen in the production of spots in wheat fields," by P. L. Gainey and M. C. Sewell, *Jour. Agr. Res.* 45: 129–148 (1932). "Nonfertile" has reference to the area immediately surrounding the "fertile" spot. The expressions "fertile" and "nonfertile" should be taken literally only when any particular pair of samples is compared. The "nonfertile" soil of one field may have been actually more productive than the "fertile" soil of another field.

[3] This term is used as employed by S. Winogradsky and Mme. J. Ziemiecka in "Etudes sur la microbiologie du sol. Sur le pouvoir fixateur des terres," *Ann. Inst. Pasteur Mem.* 3, 42: 36–62 (1918).

[4] Gainey, P. L., Sewell, M. C., and Meyers, H. E. Nitrogen—the major cause in the production of spotted wheat fields. Kans. Agr. Exp. Sta. Tech. Bul. (In press).

actual observed concentration of nitrates in the more productive samples was frequently so low as to cast doubt upon such an assumption. It should be noted, however, that there is no unanimity of opinion expressed in the literature as to the concentration of nitrates necessary to cause injury to Azotobacter, and the recorded minimum injurious concentrations vary widely. In view of the well-established fact that the organisms of this group vary markedly in many of their characteristics, it seemed plausible that the variations observed

TABLE 1

Nitrate content and density of Azotobacter flora in fertile and nonfertile soil

1930					1931				
Soil Number	NO_3 in soil		Colonies Azotobacter		Soil number	NO_3 in soil		Colonies Azotobacter	
	Nonfertile	Fertile	Nonfertile	Fertile		Nonfertile	Fertile	Nonfertile	Fertile
	p.p.m.	*p.p.m.*				*p.p.m.*	*p.p.m.*		
2	Tr.	6.0	74	64	1	10.0	10.0	19	3
3	2.0	23.0	22	36*	5	9.7	50.7	19	10
4	0	11.0	110	121*	7	7.7	99.3	21	16
6	3.0	3.0	30	29	8	7.8	342.7	175	75
8	4.0	16.0	80	55	10	6.7	86.6	50	40
11	2.0	20.0	95	74	12	9.3	158.2	95	50
16	2.0	5.0	21	9	13	9.6	140.2	75	45
18	Tr.	38.0	263	190	14	4.5	10.5	90	18
19	Tr.	14.0	530	176	16	6.2	64.8	1	3*
21	0	10.0	48	29	17	16.5	22.3	5	1
23	Tr.	10.0	510	430	20	5.3	156.3	59	7
24	0	Tr.	34	2	25	4.8	25.1	35	45*
25	0	15.0	92	90	27	3.8	32.7	2	2
27	0	0	68	27	28	4.8	47.1	49	26
28	0	13.0	6	1	29	2.5	6.4	32	10
29	Tr.	Tr.	115	115	31	4.7	109.0	90	70
31	Tr.	Tr.	140	155*	32	3.1	24.4	105	105
35	0	Tr.	1000	550	33	3.9	21.8	3	2
37	Tr.	6.0	148	127	34	1.4	5.8	50	6
39	0	12.0	14	2	36	3.2	4.0	56	33
40	0	52.0	125	190*	40	3.0	5.9	160	155
43	0	Tr.	79	36					
Ave.	0.6	11.5	164	114		6.1	67.8	54	33

* Instances in which the colony count was higher in the fertile sample.

in tolerance to nitrates, even though the nitrate content was relatively low, might be explained on a basis of strain variation.

FIELD OBSERVATIONS

Differences in the density of the Azotobacter population were determined as suggested by Winogradsky,[5] except in the type of medium employed. Freshly

[5] Winogradsky, M. S. 1925 Etudes sur la microbiologie du sol. I. Sur la méthode. *Ann. Inst. Pasteur* 39: 299–354.

prepared poured plates of washed mannite agar were inoculated by sprinkling a weighed quantity of the soil over the surface and these were incubated at 28° to 30°C. The Azotobacter colonies developing were counted after 3 to 5 days.

In table 1 are presented the data relative to the number of Azotobacter colonies developing from, and the nitrate content of, the soils examined in 1930 and 1931. Only data from the soils examined which exhibited Azotobacter by this method are included. Azotobacter were not detected in either sample of many soils. It is evident from these data that even though differences in Azotobacter and in nitrate nitrogen contents are not marked, yet a fairly consistent inverse ration between the two can be noted.

Relative to the nitrate content, attention should be directed to the fact that wheat was growing on all these soils and that the samples were taken at about the time the grain was making its maximum growth, hence, a heavy draught was being made upon the soil's store of soluble nitrogen. Furthermore, at the same time the soil samples were taken, the above-surface plant growth was collected from a measured area, dried, weighed, and analyzed for total nitrogen. For the season of 1930, 4.8 and for 1931, 4.2 times as much nitrogen had been removed from the soil by the aerial growth on the fertile area as had been removed by the corresponding growth upon an equal nonfertile area. If it is assumed that this nitrogen was absorbed in the form of nitrates it is possible that variations in the concentrations were much more marked somewhat earlier than at the time the samples were taken. Furthermore, the source of the higher nitrate content, in case of the fertile areas, has been traced to the nitrification of urea in urine deposited by cattle grazing upon the growing grain during late fall and early spring. Since the samples studied were collected during a period beginning shortly after active growth started in the spring and ending before flowering of the plants, the existence of high nitrate contents must have been of short duration, at least in many instances. The low nitrate content, even of the fertile samples, for 1930 indicates the existence of unfavorable conditions for nitrate formation and hence its consumption by the growing plants as rapidly as formed.

The counts recorded in table 1 represent the colonies developing from the particles of soil contained in 1 or 0.5 gm. soil and do not represent individual organisms; hence, the relatively low counts. Because of the heavier growth of wheat on the fertile soil, the moisture content of the nonfertile sample was almost invariably slightly higher. Since the weighed inoculum was not corrected for moisture, the actual quantities of soil used as inoculum were slightly less in the case of the nonfertile soil. This condition and also the tendency of the more moist soil to form larger particles would result in colony counts lower than actually obtained in case of the nonfertile soil and would, to that extent, minimize observed differences between the fertile and the nonfertile samples.

Inasmuch as the soils were obtained from a wide variety of conditions and as only one pair of samples was collected from any one field, it was thought well to verify the variations in the Azotobacter count between fertile and non-

fertile soils by collecting a number of samples from the same field. In 1933, 26 pairs of samples were taken from the same field. The data relative to these samples are recorded in table 2.

Although the colony counts recorded in either table 1 or 2 are not markedly in favor of the less fertile of a pair of samples, they tend in that direction so consistently that there is little doubt of the existence of an actual difference. Of 69 samples here included only 9 gave higher counts in the more fertile of the pair.

TABLE 2

Density of Azotobacter flora in fertile and nonfertile samples of soil all from same field

SAMPLE NUMBER	COLONIES AZOTOBACTER		SAMPLE NUMBER	COLONIES AZOTOBACTER	
	Nonfertile	Fertile		Nonfertile	Fertile
1	92	3	15	27	26
2	167	160	16	112	93
3	106	92	17	64	38
4	169	70	18	110	154*
5	123	56	19	165	80
6	120	64	20	111	53
7	27	31*	21	47	5
8	107	88	22	59	28
9	98	38	23	87	75
10	188	153	24	132	117
11	210	170	25	94	74
12	3	2	26	101	84
13	5	8*			
14	66	45	Average.....	100	70

* Instances in which the colony count from fertile samples exceeded that from the nonfertile.

$$\text{Ratio 1930 } \frac{\text{Nonfertile}}{\text{Fertile}} = 1.44$$

$$\text{Ratio 1931 } \frac{\text{Nonfertile}}{\text{Fertile}} = 1.64$$

$$\text{Ratio 1933 } \frac{\text{Nonfertile}}{\text{Fertile}} = 1.43$$

LABORATORY OBSERVATIONS

To gain information relative to the concentration of nitrates and the length of time necessary appreciably to affect the Azotobacter population of soils of the type under study, laboratory experiments were conducted on two different soils, S and X.

The soils were brought to the laboratory, passed through a 2-mm. sieve, and kept in moist condition for some time in order that more or less of a biological equilibrium might be reached under laboratory conditions. A sufficient number of samples were then prepared to permit of the analyses indicated in

tables 3 and 4. To these samples were added the indicated quantities of nitrate and urea nitrogen, along with sufficient water to bring the X samples to 50 per cent saturation (30 cc. per 100 gm. soil) and the S samples to 33 per cent saturation (27 cc. per 100 gm. soil). Larger quantities of moisture could not

TABLE 3

Influences of the addition of nitrate and urea nitrogen upon the Azotobacter colony count in soils S and X

NITROGEN ADDED TO SOIL			COLONY COUNT* OF AZOTOBACTER AFTER VARYING PERIODS OF TIME						
Form	Soil	Amount	0 wks.†	2 wks.	4 wks.	8 wks.	12 wks.	16 wks.	20 wks.
		p.p.m.							
KNO_3	X	0	65	71	65	84	62	68	31
KNO_3	X	50	55	86	71	82	60	88	63
KNO_3	X	150	61	88	67	74	67	71	40
KNO_3	X	500	72	91	60	53	78	79	38
Urea	X	50	82	95	57	51	73	68	53
Urea	X	150	81	72	83	82	85	87	36
Urea	X	500	77	81	50	48	69	67	19
KNO_3	S	0	53	74	60	68	53	49	...
KNO_3	S	50	57	79	55	66	34	43	...
KNO_3	S	150	49	59	41	19	10	14	...
KNO_3	S	500	31	6	0	0	0	0	...
Urea	S	500	—	79	49	0	0	0	...

* Count from 3-day-old undiluted plates in case of soil X and diluted plates in case of soil S.
† Count one day after treatment.

TABLE 4

Influence of nitrate nitrogen upon the colony count of Azotobacter in soils S and X

NITROGEN ADDED TO SOIL AS KNO_3	SOIL	COLONY COUNT* OF AZOTOBACTER AFTER VARYING PERIODS OF TIME			
		0 wks.†	2 wks.	4 wks.	8 wks.
p.p.m. N					
0	X	26	21	34	14
500	X	23	14	18	9
1000	X	25	9	9	6
2000	X	18	9	5	1
0	S	61	79	40	26
500	S	87	9	7	2
1000	S	39	0	0	0
2000	S	32	1	0	0

* Count on diluted plates after 3 days of incubation.
† Sampled one day after treatment.

be employed in either case because of puddling. In these tablets are also recorded the Azotobacter density following various treatments after varying intervals of time. During incubation the moisture content was kept constant by frequent additions of water.

Because of the high initial Azotobacter content of these soils it was necessary to resort to some method of diluting before plating. This was accomplished by thoroughly mixing 1 part of the soil in question with 9 parts of an Azotobacter-free soil. The diluting soil had previously been passed through a 20-mesh sieve but retained by a 40-mesh sieve. This eliminated the finer particles, which apparently aided in the coalescing of colonies, and facilitated the formation of discrete colonies.

If the NO_3 ion is responsible for the decrease in the Azotobacter population here recorded, the question naturally arises as to how the decrease in Azotobacter is to be accounted for when nitrogen in the form of urea is added. Suffice it to say that all these soil samples were analyzed for nitrate nitrogen, and the urea nitrogen was found to be transformed into nitrate nitrogen very rapidly. The following data relative to the NO_3—N content of soil X after 4 weeks illustrate this:

Treatment p.p.m. N	*NO_3-N recovered p.p.m.*
0 as KNO_3	43.4
50 as KNO_3	114.6
150 as KNO_3	210.2
500 as KNO_3	559.6
50 as urea	103.7
150 as urea	233.7
500 as urea	576.8

It is evident from the data presented in table 3 that the injurious effect of the application of nitrogen was slightly delayed in the case of urea; this fact indicates the necessity for a sufficient lapse of time to enable nitrification to take place. Nitrification did not take place so rapidly in soil S as in soil X. Aside from this slight delay in accumulation, the nitrate contents of the samples treated with urea nitrogen were, on the average, as high as those treated with nitrate nitrogen.

The following three points seem worthy of special note in connection with the data recorded in tables 3 and 4:

There was a marked difference in the sensitivity to nitrates of the Azotobacter in the two soils, or else the environmental conditions were such as to render the organisms in soil S less resistant to injury.

A decrease in the Azotobacter density, as here determined, became evident in a relatively short period of time; there were some indications of such an effect within 24 hours and a very marked effect within 2 weeks.

The concentration of nitrates, expressed in parts per million soil, necessary to reduce appreciably the density of the Azotobacter population may vary in different soils but is certainly not very high for soil S under the conditions existing in these experiments.

PURE CULTURE STUDIES

A large number of cultures, all isolated from local soils, have been studied with reference to their tolerance of nitrates. Under suitable conditions all these cultures produced a typical black pigment and for this reason have been

tentatively classed as strains of *Azotobacter chroococcum*, though no effort has been made to verify this grouping. All isolations and purification of cultures were made on mannite agar to which no nitrogen was added. With the exception of the early cultivation of series I all cultures were grown on mannite agar slants, prepared from washed agar-agar (100 gm. powdered agar-agar suspended in 4 liters distilled water and the water renewed every day or two for a period of 2 weeks) to which the different nitrates were added to give the desired concentrations of NO_3—N. The washing process reduced the total nitrogen content of the agar from 25–30 p.p.m. to 5–8 p.p.m. Such an agar to which no nitrogen was added is designated as "nitrogen-free," and although this is technically incorrect the nitrogen present must have been in a highly complex insoluble form and, therefore, probably not readily available to Azotobacter. Cultures were transferred every 3 or 4 days and incubated at 28° to 30°C. Observations of growth and pigmentation were recorded after 3, 7, and sometimes 14 days. It required 2 to 4 days for cultures to attain their maximum visible growth, and much longer periods between transfers at high nitrate concentrations were decidedly injurious.

The composition of the nitrogen-free medium employed was as follows:

Dipotassium phosphate	2.50 gm.
Magnesium sulfate	0.20 gm.
Sodium chloride	0.20 gm.
Calcium chloride	0.05 gm.
Ferric chloride, 10 per cent solution	1 drop
Mannite	20.00 gm.
Agar-agar, washed	20.00 gm.
Distilled water	1000 cc.

After the medium was autoclaved to dissolve the agar-agar, the salts, a few drops of phenolphthalein indicator, and sufficient *N* NaOH to give a faint, but permanent, pink color were added. The reaction of such a medium upon standing was always acid to phenolphthalein and alkaline to brom-thymol blue, hence in the vicinity of pH 8.0, since sufficient buffering effect was produced by the phosphate to prevent any marked change in pH.

Series I. Series I consisted of 13 cultures isolated and grown on unwashed agar containing 0, 1, 10, 25, 50, 75, and 100 p.p.m. KNO_3—N for a period of 190 days. The only consistent differences observed were that cultures 8, 20, 40, and 36 were a little slow in developing on 100 p.p.m. KNO_3—N following the first few transfers, and that cultures 5 and 32 exhibited a similar retarded growth at 50 and 75 p.p.m. and failed to grow at 100 p.p.m. KNO_3—N when inoculated from the nitrogen-free medium but grew normally when inoculated from the 75 p.p.m. N medium. These are the only cultures that received a preliminary cultivation on unwashed agar.

After a period of 70 to 73 days on the unwashed agar medium each culture was transferred to washed agar containing the corresponding concentrations of KNO_3—N. They were continued on these media for periods of 116 to 123

days. The only consistent difference observed was that culture 40 grew poorly on the nitrogen-free medium for a short time.

These 13 cultures were left on nitrogen-free agar for a period of 7½ months, after which 9 were recultured and tested for purity. These, together with two new isolations, were transferred to washed agar containing 0, 100, 500, 1000,

TABLE 5

Effect of various concentrations of KNO_3–N upon the growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.									
	0	100	500	1000	3000		4000		5000	
	Growth	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth
5	4x	4x	4x*	1x–3x	0	0	0	0	0	0
					1000	0	1000	0	1000	0
8	4x	4x	4x	4x	0	0	0	0	0	0
					1000	4x†	3000	1x–4x	3000	0
									4000	1x–4x
12A	4x	4x	4x	4x	0	4x	0	4x	0	4x
12B	4x	4x	4x	4x	0	4x	0	4x	0	0‡
									4000	4x
31	4x	4x	4x	4x	0	0	1000	0	1000	0
					1000	4x*				
36	4x	4x	4x	4x	0	4x†	0	0	0	0
							3000	1x–4x	4000	0
A	4x	4x	4x	4x	0	4x†	0	0	0	0
							3000	1x–4x	4000	0
EA	4x	4x	4x	4x	0	4x†	0	0	0	0
							3000	4x	3000	0
									4000	4x†
EB	4x	4x	4x	4x	0	4x†	0	0	0	0
							1000	0‡	1000	0‡
S	4x	4x	4x	4x	0	0	0	0	0	0
							1000	0	1000	0
X	4x	4x	4x	4x	0	4x†	0	1x–4x	0	0
									3000	0
									4000	1x–4x

* Markedly retarded.

† Some retardation.

‡ Died after few transfers.

(In this and all subsequent tables 4x = normal; 2x = one half normal growth; etc.)

3000, and in some instances 4000 and 5000, p.p.m. KNO_3—N. Because of difficulties sometimes encountered in inducing growth on the higher concentrations and also because of an accidental failure to transfer cultures regularly during one period of 4 weeks, at which time every culture completely lost its vigor at concentrations varying from 500 to 3000 p.p.m. N and had to be

started again from the N-free culture, there was considerable irregularity with respect to the time the different cultures were grown at various concentrations. Where repeated transfers from the N-free medium to higher concentrations failed to grow, some lower concentration was used as the source of inoculum. In this way normal growth of Azotobacter frequently could be obtained at much higher concentrations than when cultured only from the N-free culture. Also, in some instances where growth of a culture was very slight after 3 to 4 days and no perceptible growth appeared when the culture was transferred, if the original culture was allowed to grow for 7 to 10 days and then was transferred, growth might result. In a few instances fair or even normal growth would take place for a period of time at relatively high concentrations and then gradually die out. On other slants growth at high concentrations would be slow or feeble for a time, after which it became normal.

TABLE 6

Effect of different concentrations of $NaNO_3$—N upon growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATIONS OF NITROGEN, P.P.M.								
	0	500	1000	3000		4000		5000	
	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth
S	4x	4x	4x	0	0				
				1000	1x–4x*				
X	4x	4x	4x	0	0†				
				1000	2x–4x				
12B	4x	4x	4x	0	4x	0	2x–4x	0	2x–4x
8	4x	4x	4x	0	4x				

* Grew irregularly for a few weeks then died.

† Died after a few transfers.

The term "normal" as here used has reference solely to the mass of visible growth occurring on agar slants, growth on the nitrogen-free agar being taken as standard. It might be suggested that morphologically many of the cultures could not be regarded as normal. Many irregular and bizarre forms were observed, particularly in the $Ca(NO_3)_2$ media and in higher concentrations of other forms of nitrate. The relative growth of these cultures on agar containing varying concentrations of NO_3—N is recorded in table 5.

It is worthy of note that culture 5 which would not grow on agar containing 100 p.p.m. NO_3—N originally was finally induced to grow normally at 500 and irregularly at 1000 p.p.m. NO_3—N.

In order to obtain evidence that the injurious effect of nitrates is due to the NO_3 ion and not to the cation, other salts of HNO_3, including $NaNO_3$, $Ca(NO_3)_2$, and NH_4NO_3, were substituted for KNO_3. The data from series I grown in the

presence of these forms of nitrogen are presented in table 6, 7, and 8. From these data the following points appear worthy of special attention:

A marked variation in the tolerance of different strains of Azotobacter to the nitrate ion is evident. For example, cultures 5 and S failed to grow at 3000 p.p.m. NO_3—N, the former

TABLE 7

Effect of different concentrations of $Ca(NO_3)_2$—N upon growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.					
	0	500	1000		3000	
	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth
S	4x	4x	0	0	0	0
			500	0*	1000	0
X	4x	1x–4x	0	0	0	0
			500	0*	1000	0
EA	4x	4x	0	0*	0	0
			500	1x‡	500	0
12B	4x	4x†	0	0	0	0
			500	0*	500	0
8	4x	0*	0			

* Died after a few transfers.
† Retarded following first few transfers.
‡ Growth questionable.

TABLE 8

Effect of different concentrations of NH_4NO_3—N upon growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.						
	0	250	500	1000	1500	3000	
	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth
S	4x	4x	4x	4x	2x–4x	0	0
						1000	0
X	4x	4x	4x	4x	4x	0	1x–4x*
12B	4x	4x	4x	4x	4x	0	0
						1500	1x–3x
8	4x	4x	4x	4x	1x–4x	0	1x–3x*

* Died after a few weeks.

appearing especially susceptible to injury. On the other hand, cultures 8, 12A, 12B, EA, and X all grew at 5000 p.p.m. NO_3—N, 12A growing normally at this concentration even when brought directly from the N-free medium.

Many strains of Azotobacter can be induced, by gradual adaptation, to grow normally in concentrations of nitrates in which they otherwise will not grow.

TABLE 9

Effect of different concentrations of KNO_3—N upon the growth of pure cultures of Azotobacter—series II

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.										
	0	100	500	1000	2000	4000		4500		5000	
	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth
BB	4x	4x	4x	4x	4x	0	4x*	4000	4x	4000	4x
IIA	4x	4x	4x	4x	4x	0†	1x–4x*	4000	0	4000	0
IIB	4x	4x	4x	4x	4x	0†	4x*	4000	1x–4x	4000	1x–4x
IIC	4x	4x	4x	4x	4x	0†	1x–4x	4000	1x–4x	4000	1x–4x
IIIC	4x	4x	4x	4x	4x	0	1x–4x	4000	1x–4x	4000	1x–4x
IIID	4x	4x	4x	4x	4x	0†	1x–4x*	4000	1x–4x	4000	0‡
IIIE	4x	4x	4x	4x	4x	0	1x–4x*	4000	0‡	4000	0‡
IIIF	4x	4x	4x	4x	4x	0	1x–4x	4000	0‡	4000	0‡
IID	4x	4x	4x	4x	4x*	0†	1x–4x*	4000	0‡	4000	0‡
IIE	4x	4x	4x	4x	4x	0	1x–4x*	4000	1x–4x	4000	1x–4x
IIIA	4x	4x	4x	4x	4x	0†	1x–4x*	4000	1x–4x	4000	1x–4x
IIIB	4x	4x	4x	4x	4x	0	1x–4x*	4000	1x–4x	4000	1x–4x

* Growth retarded following first few transfers.

† Transferred from 0 after 3–4 days, growth failed; transferred from 0 after 10 days, growth took place.

‡ Died after a few transfers.

TABLE 10

Effect of different concentrations of $Ca(NO_3)_2$—N upon the growth of pure cultures of Azotobacter—series II

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.					
	0	100	500	1000	2000	4000
	Growth	Growth	Growth	Growth	Growth	Growth
BB	4x	4x	4x	2x–4x	2x–3x	0
IIA	4x	4x	4x	2x–4x	1x–4x	0
IIB	4x	4x	2x–4x	2x–4x	2x–4x	0
IIC	4x	4x	2x–4x	1x–4x	1x–2x	0
IIIC	4x	4x	2x–4x	1x–4x	0x–1x	0
IIID	4x	4x	4x	4x	0x–1x	0
IIIE	4x	4x	2x–4x	2x–4x	1x–4x	0
IIIF	4x	4x	1x–4x	1x–4x	0x–4x	0
IID	4x	4x	3x–4x	2x–4x	1x–4x	0
IIE	4x	....	2x–4x	2x–4x	1x–3x	0
IIIA	4x	4x	2x–4x	2x–4x	1x–3x	0
IIIB	4x	4x	2x–4x	1x–4x	0x–1x	0

TABLE 11

Effect of different concentrations of NH_4NO_3—N upon the growth of pure cultures of Actobacter—series II

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.													
	0	50 (100)*	250 (500)	500 (1000)	1000 (2000)	2000 (4000)	2500 (5000)		3000 (6000)		3500 (7000		4000 (8000	
	Growth	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth
BB	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	4x	0	0
													1000	0
													2000	0†
IIA	4x	4x	4x	4x	4x	4x	2000	4x	2000	1x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIB	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	0†	0	0
													1000	0
													2000	0†
IIC	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	4x	0	0
													1000	0
													2000	0†
IIIC	4x	4x	4x	4x	4x	4x	2000	2x–4x	2000	2x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIID	4x	4x	4x	4x	4x	4x	2000	4x	2000	2x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIIE	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIIF	4x	4x	4x	4x	4x	4x	2000	1x–4x	2000	1x–4x	2000	0	0	0
													1000	0
													2000	0†
IID	4x	4x	4x	4x	4x	4x	2000	4x	2000	2x–4x	2000	2x–4x	0	0
													1000	0
													2000	0†
IIE	4x	4x	4x	4x	4x	4x	2000	3x–4x	2000	2x–4x	2000	0†	0	0
													1000	0
													2000	0†
IIIA	4x	4x	4x	4x	4x	4x	2000	2x–4x	2000	2x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIIB	4x	4x	4x	4x	4x	4x	2000	4x	2000	2x–3x	2000	1x–4x	0	0
													1000	0
													2000	0†

* Values in parenthesis are total nitrogen.

† Died after a few weeks.

There is some indication that the sensitivity or tolerance to nitrate of pure cultures is associated with a similar sensitivity or tolerance of mixed cultures in soil. Cultures S and X were isolated from the corresponding soils. Soil S has already been described as containing a much more sensitive Azotobacter flora than X. A similar variation was observed in the pure cultures S and X.

No significant difference was noted as to the effect of the various nitrates except the apparently more toxic action of $Ca(NO_3)_2$. This difference is believed to be due, however, to the removal of phosphorus from solution in the presence of the high calcium content rather than to the nitrate ion.

TABLE 12

Effect of different concentrations of KNO_3—N upon the growth of pure cultures of Azotobacter—series III

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.						
	0	100	500	1000	2000	4000	
	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth
1	4x	4x	4x	4x	4x	0	1x–4x
2	4x	4x	4x	4x	4x	0	1x–4x
3	4x	4x	4x	4x	4x	0	0
						2000	0
4	4x	4x	4x	4x	2x–4x	0	0
						2000	0*
5	4x	4x	4x	4x	4x	0	1x–4x
6	4x	4x	4x	4x	4x	0	4x
7	4x	4x	4x	4x	4x	0	1x–4x†
8	4x	4x	4x	4x	4x	0	1x–4x†
9	4x	4x	4x	4x	4x	0	1x–4x
10	4x	4x	4x	4x	4x	0	0
						2000	0
11	4x	4x	4x	4x	2x–4x	0	0
						2000	0
12	4x	4x	4x	4x	4x	0	2x–4x†
13	4x	4x	4x	4x	4x	0	1x–4x†
14	4x	4x	4x	4x	2x–4x	0	0
						2000	2x–4x
15	4x	4x	4x	4x	4x	0	4x†

* Died after a few weeks.

† Growth retarded following first few transfers.

Series II. Series II consisted of 12 cultures all isolated, purified, and immediately transferred to varying concentrations of nitrate nitrogen in the form of KNO_3, NH_4NO_3, and $Ca(NO_3)_2$. After a period of 18 weeks on concentrations up to and including 4000 p.p.m. KNO_3—N, efforts were made to grow the cultures at 4500 and 5000 p.p.m. N by transferring them from the 4000 p.p.m. culture to the higher concentrations. Similarly, after 16 weeks on concentrations up to and including 4000 p.p.m. NH_4NO_3—N, efforts were made to grow

cultures at 5000, 6000, 7000, and 8000 p.p.m. of the same forms of nitrogen. Summaries of observations as to growth are recorded in tables 9, 10, and 11.

In this series, as in the first series, differences in tolerance to NO_3 are evident, though perhaps not so marked as in the former series. Also the cultures all appear to be less tolerant of NO_3 in the form of $Ca(NO_3)_2$ than other forms; however, as already suggested this difference was probably due to the lack of available phosphorus. Here again, by gradual adaptation, cultures could be induced to grow at much higher concentrations of NO_3 than they would tolerate normally.

Series III. A third group of cultures was isolated, purified, and immediately cultured upon agar containing varying concentrations of NO_3—N. These were grown for a period of 95 days, during which time efforts to induce the growth of 5 of the 15 cultures on 4000 p.p.m. NO_3—N failed completely; 1 culture grew normally; and the remaining 9 grew somewhat irregularly. At a concentration of 2000 p.p.m. N, 3 of the 5 cultures that failed to grow at 4000 p.p.m. N grew irregularly.

A fourth series of 24 cultures has been under observation for some time, one of which persistently refuses to grow at 100 p.p.m. N and several at 1000 p.p.m. N when cultured from N-free medium. Many of the cultures grew normally at 4000 p.p.m. N when transferred from the N-free medium, and several are now growing normally at 5500 p.p.m. KNO_3—N.

DISCUSSION

Field and laboratory observations on the effect of nitrates upon the Azotobacter flora of soils tend to support the recorded observations that relatively high concentrations in a soil containing Azotobacter tend to cause a decrease in the density of the Azotobacter population. The concentration necessary to bring about this effect appears to vary rather widely with different soils, and in some soils is not particularly high.

Observations made on 65 pure cultures of Azotobacter isolated from local soils has shown conclusively that there is a marked variation in the sensitivity of different strains to nitrate nitrogen. That this sensitivity is due to the NO_3 ion and not to the cation is indicated by the fact that the same injurious effect is produced by potassium, sodium, and ammonium nitrate. That the nitrogen alone is not responsible for the phenomenon is indicated by the fact that upon the addition of nitrogen in the form of urea, the injurious effect is not evident until sufficient time has elapsed to enable the processes of nitrification to transform the urea nitrogen into nitrate nitrogen; also, by the fact that the injurious effect of ammonium nitrate is not proportional to the total but rather to its NO_3—N content.

With the toxic action of the NO_3 upon pure cultures established, it is not necessary to postulate an antagonistic action of other organisms stimulated by the nitrate to account for the reduction in Azotobacter population following an application of nitrate nitrogen to a soil. Some evidence is submitted in

connection with soils S and X in support of the view that the variation in sensitivity of the Azotobacter of different soils may be associated with the sensitivity of the particular strains inhabiting the soil in question.

Relative to the apparently much lower concentration of NO_3 that proved toxic to Azotobacter in soils, as compared with that necessary in case of pure cultures, attention should be directed to the fact that the nitrate content of soils is always recorded on a dry soil basis. Since nitrates are very soluble, such salts are probably found dissolved in the soil moisture. The actual effective concentration, therefore, as far as the Azotobacter are influenced, would vary inversely with the moisture content of the soil and in the data here presented would be from 3 to 20 times the concentration calculated upon a dry soil basis. The fact should also be kept in mind that all the pure culture studies here recorded were made upon mannite agar. Just what influence the colloidal agar-agar exerted upon the concentration of NO_3 tolerated by Azotobacter is problematic. Comparisons between the concentrations tolerated in liquid cultures and in agar cultures are under way.

Since a relatively low concentration of NO_3—N has been found toxic in soil and since the speed of the action, as observed in soil S, may be rapid, it is not impossible that the differences observed in the density of the Azotobacter population in "fertile" and "nonfertile" soils were due to differences in the NO_3—N content that existed in the pair of samples at some time. In view of the relative ease with which increased tolerance can be induced in the laboratory, however, it is difficult to see why a natural tolerance to the concentrations of NO_3 ordinarily encountered in the more fertile soils, or in soils fertilized with nitrate nitrogen, is not developed.

ON THE FORMATION OF STRUCTURE IN SOIL: II. SYNTHESIS OF AGGREGATES; ON THE BONDS UNITING CLAY WITH SAND AND CLAY WITH HUMUS

D. I. SIDERI

Institute of Fertilizers and Soil Science, Moscow, U. S. S. R.

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In this paper a new theory of structure formation based on the capacity of soil colloids to form phases of the aggregate state is proposed.

Quartz sand mixed with fine particles of colloidal clay (in the state of sol or gel) assumes the structural form of clay. "Intergrowth aggregates" are formed as a result of the similarity of the crystalline periods of clay and quartz. The tenacity of these intergrowths is so great that aggregates prepared from highly hydrated Na-clay and perfectly pure quartz sand show a very remarkable resistance to water and do not differ from similar aggregates prepared from Ca-clay.

Humus is irreversibly adsorbed by clay, and this raises the tenacity of the aggregates. This increase of water-resistance, however, is not observed for every clay in the same degree; in fact, some clays are not in the least affected by humus. The binding of humus with clay is due to the selective orientation of humus particles on clay according to the type of a non-homogeneous mesomorphous system. The aggregation of colloid particles causes the deformation of ion layers around them: a compression of the ion layer takes place. As a result, these differences, conditioned by the nature of the absorbed cations, are levelled out.

Calcium influences the particles of those colloids which water affects more intensely, i.e., loosely packed particles (unstable systems) or particles irregularly distributed.

The first investigators of the problem of soil structure attached great importance to clay and humus. Schloesing (36) thought that these substances were able to cement the mineral elements in soil. The ideas of Schloesing were later developed further by Dumont (8), who suggested that the important properties of soil depend on colloid envelopes: the active colloidal material being a jelly-like or amorphous mass, enveloping solid mineral grains. This idea was afterwards supported by Hall (18), who held the opinion that since the colloidal envelope has no defined external surface it can be considered as surrounding the soil particles in a series of cells with a gradually increasing hydration, so that there is a gradual transition from the solid grain to the solution. The colloidal envelopes alter essentially the properties of mineral particles and render the soil structure cellular and reticular (19). Very

similar conclusions are suggested by Comber (5) and by Haines (17), who demonstrated by their experiments how greatly the colloidal envelope affects the properties of mineral grains and how far it aids aggregation.

There exists, however, another point of view, according to which the ability to aggregate depends on the surface water film. Manegold (26) determines the force of cohesion on the basis of the properties of a liquid ring lying between two spheres of equal size, if all excess liquid is eliminated. According to Nutting (29), the thin absorption film binds the particles into aggregates: in his words,". . . the cohesive pressure may mount to a respectable figure, depending largely upon the size and shape of the grains." According to Norton (28), the thin water film plays a decisive part in the formation of the structure of clays. Clay particles attract strongly to their surface their water films, which they have in common with the adjoining particles. This phenomenon determines cohesion and lubrication simultaneously. Norton (27) thinks it possible to determine the nature of surface films by studying the mechanism of shrinkage during drying and the curves of shrinkage.

Wetting with water loosens the bonds between the particles. In this case swelling is understood as a sufficient increase in the thickness of the layer of adhesion that it reaches the value of the radius of the sphere (grain). The liquid rings between the spheres disappear during swelling and, because of this, are lost (26). According to Derjaguin (7) water lowers the friction between the crystalline lamellas. A fine-structural breaking-up of the tri-dimensional periodic structure of the crystal into a one-dimensional and a two-dimensional structure of the mesomorphous type takes place under the influence of water. For bromphenanthrene sulfonic acid this has been established by Rinne (33); for clays, by the author.

The aggregates in soil, however, do not always break up after being wet. There exist tenacious structures which are not affected by distilled water (35), and it has been found by the author that such structures are resistant to alkalis as well. The present work was undertaken for the elucidation of the phenomena of structure formation.

MATERIALS

Pure quartz sand was used for the synthesis of aggregates. The sand was ground in a ball-mill and then was freed from contamination by being washed several times with a boiling HCl solution. The acid was washed off first with tap water then with distilled water. After this, the sand was fractionated by means of elutriation. The elutriated sand was heated for several hours in a muffle-furnace, cooled in a desiccator, and stored in a glass container fitted with a ground stopper.

The clay suspensions were prepared from various rocks belonging mostly to Quaternary deposits.[1] The method of preparation of these clay suspensions,

[1] The rock samples were obtained from the collection of Professor Mirchink; two samples of clay from carbon and Jura deposits were taken from the shafts of the Moscow Underground Railway.

the data on their mineralogical composition, and a description of the humates used for these experiments have been given in part I (37).

ADSORPTION OF CLAY ON QUARTZ

Water films may stabilize the weak skeleton of sand if the excess water will flow out of the sand mound under the influence of gravity. The cohesion between the grains of sand will increase if their water film is enriched by some soluble substance, e.g., clay. Will there be a condensation of the dissolved substance on the surface of the quartz grains in the shape of an adsorbed layer, or will the layer of adsorbed molecules serve as a point of departure for chains of oriented molecules, extending into the mass of the binding substance (23, 24, 25), i.e., will a layer of adhesion determining the value of the stress necessary for disrupting the bond be formed?

Adsorption of clay by quartz has not been studied heretofore. Observations point to the possibility of the clay's condensation on the quartz in the shape of very tenacious pellicles which are not dissolved by water. It is almost impossible to obtain clay-free sand in the process of the texture analysis of soil even if the dispersion of the colloids is complete.

Anderson, et al. (1) have found, that the separation of soil into colloids and non-colloids is impossible, since colloidal material cannot be freed from fine mineral particles.

Adsorption of clay on quartz may take place on the basis of the similarity of the crystalline periods of quartz and clay. In silica the layer of the tetrahedrons of silicon oxygen has the shape of a hexagon with the length $a_0 = 5.2$ Å. Kaolinite is made up of silicon-oxygen layers, oriented in a hexagonal pattern, the distance a_0 equaling, according to the measurements of Gruner (16), 5.14 Å. A similar pattern, a hexagonal ring of three silicon oxygen tetrahedrons bound together, has been found for montmorillonite.

Adsorption was observed in almost every sample of clay taken by us. Quartz sand with a grain-size of 20–50μ was used for the experiments. The container was thoroughly cleansed with chromium mixture, and the pipette was washed with clay sol. The volume of the clay suspension reached 25–50 cc. for 5–10 gm. of sand. The time of adsorption was 1 hour. After adsorption, the suspension was filtered through a filter, which did not let the sand pass (the filtrate was examined for sand under the microscope). The value of the adsorption was determined by the final concentration of the solution. It was found that after the sand was immersed in the solution the equilibrium concentration changed somewhat. To determine whether the adsorption was actually irreversible, the powdered quartz, after the liquid was decanted, was divided into two equal parts. In one of these we determined the irreversibility of the freshly deposited clay layer; in the other, the irreversibility of this layer after a preliminary drying at 36°C. In both cases the irreversibility of adsorption was established by means of a thorough and prolonged washing of the powder with distilled water. The washing was continued until no turbidity

was apparent. Usually this washing took 3 or 4 days, the sand being left under water overnight, and the washing was terminated if on the next morning no turbidity appeared after mixing (without the use of a rod). This method was strictly adhered to in all the following experiments. After the washing, the layer of adsorbed clay was dissolved in a 10 per cent boiling HCl solution. These experiments showed that not all of the samples of freshly adsorbed clay pellicles had the property of irreversibility. The irreversibility of the clay layers after being dried, however, was very marked for all our samples.

TABLE 1

Adsorption of clay on quartz sand

CLAY	C*	a†	IRREVERSIBLE ADSORPTION	
			a_I‡	a_{II}§
	per cent			
1. Mokshany (cover clay, Riss)	2.44	1.0	0	0.42
2. Kobeliaki (fluvio-glacial, Würms)	....	....	0.12	0.40
3. Red clay (Carboniferous)	2.24	0.42	0	0.44
4. Zhaki (loess loam, Würms)	3.68	0.76	0	1.80
5. Ostrovy (bottom moraine, Riss)	3.25	0.64	0	0.88
6. Glivin (moraine, Mindel)	2.57	0.46	0.12	0.56
7. Riazan (moraine, Riss)	2.51	1.68	‖	‖
8. Dzekan (loess loam, Würms)	4.48	0.58	0.30	0.60
9. Alatyr (cover clay, after Riss)	2.71	0.28	0	0.80
10. Toida (cover clay, after Riss)	3.10	0.85	0.56	1.50
11. Seno (fluvio-glacial clay, Würms)	4.79	0.67	0.36	...
12. Black clay (Jura)	....	....	....	0.96

* C = equilibrium concentration in gm. per 100 cc. of solution.
† a = mgm. clay absorbed per gm. of quartz sand.
‡ a_I = mgm. freshly precipitated clay per gm. of quartz sand.
§ a_{II} = mgm. clay, after preliminary drying at 36°C., per gm. of quartz sand.
‖ Not measured.

MEASUREMENT RESULTS

Table 1 shows the results of the adsorption experiments. Since several suspensions coagulated during filtration, they have not been included.

The irregularity in the properties of the crystalline surface and the existence of active centers on this surface make precise calculations regarding the monomolecular layer impossible. Moreover, not every clay of those we examined is uniform in its mineralogic composition. The order of their adsorption values, however, suggests the possibility of a monomolecular layer. A detailed consideration of this question is not necessary for the explanation of the nature of aggregation. The bond, conditioned by the force of cohesion of the molecules of the adsorbed layer, acts within a small distance, on the order of a double layer of clay crystallites. Even in a relatively fine sand, such as was taken for

the experiment 20–50μ, a bond of this character seems to be rare. Under the microscope only rare small aggregates united by thin adhesion layers are observed in sand moistened with water. The prevailing mass of sand consists of separate grains.

The data of table 1 show the increase of irreversibility under the influence of drying. This is particularly apparent in those clays the freshly precipitated layers of which proved completely reversible. Drying assists in the accumulation of clay in the irreversible state. It is possible to raise this accumulation to a certain maximum point and, with it, the force of cohesion as well. This process is due to the fact that orientation is not limited to one or two layers of molecules but extends much farther, one layer of molecules tending to orient the next, until orientation is fixed and there is no disorientation. Side by side with the formation of the adhesion layer on the crystalline surface there may be also a grouping similar to that of the mesomorphous system under the influence of the surface water film (pl. 2, fig. 1).

ON THE BOND BETWEEN CLAY AND QUARTZ SAND

The existence of a firm bond between clay and quartz may be explained in several ways. The intergrowth of clay crystallites with quartz, similar to non-homogeneous crystalline formations definitely oriented to one another, may be formed. It is possible that the displacement of quartz by kaolinite observed under natural conditions—such as the one described by Ross and Kerr (34) for a clay from Camden, Alabama—must be classed among phenomena of this kind.

Besides crystalline intergrowth the formation of non-homogeneous aggregates is possible also along the type of the mesomorphous or the paracrystalline system. The observation of Nutting, that "aggregates of fine and coarse particles, taken together, when moist assume the properties of the fine particles," refers to this type of aggregation (pl. 2, fig. 1).

The remarkable property of sand to acquire the properties of clay has been noted by Freundlich (9) also in respect of tyxotropic phenomena. Freundlich describes a case in which the tyxotropic properties of a quartz sand were determined by the presence of an argillaceous substance.

The ability of sand to assume the form of grouping belonging to clay is explained by the similarity of their forms of orientation: fine sand that has been mixed with water, when it dries gathers, under the influence of the film of surface tension, into rows resembling the structure of the smectic phase of clay [pl. 1, fig. 10, (37)]. The smectic phase of clay possesses a partially periodical structure, the periods of which coincide with the crystalline periods of quartz. Thus the condition necessary for the occurrence of selective orientation or "épitaxie" (according to Friedel, 14) arises. The tenacity of the "intergrowth" does not depend, therefore, on the nature of the absorbed bases (cations) and of the liospheres determined by them.

The tenacity of the bond between sand and clay increases with the decrease

of grain-size. It is quite impossible to separate fine sand from clay, as was long ago indicated by Schloesing (35, p. 544) and is well known from the practice of soil texture analyses. With the increase of the size of sand grains the tenacity of the bond decreases rapidly; clay can be easily washed off coarse sand, only thin adhesion layers being left on the surface of the sand grains. Experiments were therefore arranged for demonstrating the possibility of the formation of stable ("water-tight") structures from highly hydrated Na-clay.

Mixtures of clay with quartz sand (10–20μ), containing about 15 per cent of clay, were prepared. A Na-clay sol was slowly evaporated in the incubator at 35°C. until it reached the state of a very viscous jelly-like mass. Perfectly pure sand was mixed in a porcelain dish with a weighed amount of clay, and the mixture was left to dry slowly for several days at room temperature in a place protected from dust. During this drying the mass was thoroughly mixed several times with the purpose of obtaining a uniform mixture of clay with sand.

TABLE 2

Irreversibility of Na-clays mixed with sands

CLAY	CLAY CONTENT OF INITIAL MATERIAL	CLAY CONTENT OF RESIDUE AFTER WASHING WITH WATER (INSOLUBLE CLAY)	LOSS
	*mgm.**	*mgm.**	*per cent*
1 Zhaki (loesslike loam, Würms)	142.9	129.1	9.7
2. Toida (cover clay, after Riss)	151.5	58.1	61.6
3. Kobeliaki (fluvio-glacial clay, Würms)	146.2	60.6	58.6
4. Ostrovy (bottom moraine, Riss)	146.8	68.3	53.4
5. Red clay (Carboniferous)	122.6	69.7	43.1
6. Alatyr (cover clay, after Riss)	140.8	67.7	51.9

* Per gm. of mixture.

Mixtures of sand with Ca-clay were prepared in the same manner for comparison. Ca-clays were prepared by precipitating Na-clays with a normal solution of pure $CaCl_2$ and by subsequently washing the Ca-clay precipitate many times with the same $CaCl_2$ solution. The Ca-clay precipitates were thoroughly dialyzed so that they would contain no water-soluble salts. The clear supernatant liquid after sedimentation was siphoned off.

When the weight of the mixtures of clay and sand reached a constant value, experiments were set up for the determination of the amount of firmly bound clay, i.e., clay insoluble in distilled water. Two grams of each of the mixtures of sand with Na- and Ca-clays, calculated as dry weight, was thoroughly washed with distilled water according to the technique just described until clay ceased completely to dissolve. Clay, adhering tenaciously to the grains of sand, was determined after it was dissolved with hydrochloric acid. The sediment washed with water was treated with acid on a water bath until the

clay was completely dissolved, after which the sediment was washed on a filter by a hot solution of acid. If not all the clay had been dissolved, as could be seen from the coloring, the sediment was again transferred, together with the filter, into a porcelain dish and the clay was dissolved in aqua regia. Usually the washed sediment after being heated in a muffle furnace was perfectly white.

Table 2 shows the results of the determinations of the insolubility of Na-clays mixed with sand.

A remarkable result of these experiments on synthetizing aggregates is that we were able to obtain a tenacious water-tight structure from highly hydrated Na-clays. This phenomenon is in contradiction to, and cannot be explained by, the existing conceptions regarding the nature of soil colloids and the direct participation of adsorbed Ca in the process of structure formation. It should be noted that the possibility of the formation of an isoelectric precipitate in this case is excluded when sand is mixed with clay. One might consider hysteresis in this connection, but our observations on the swelling of Na-clay dried at 100°C. show that in some cases (Toida, Alatyr) the clay is dissolved. Moreover Na-clay sols dried at 100°C. are, without exception, soluble in water.

THE ACTION OF ALKALI ON TENACIOUS AGGREGATES OF SAND AND Na-CLAY

Alkalis are considered to increase the dispersion of clay, but the general conception among soil scientists concerning the influence of OH ions is not applicable to this case (6), since we are dealing here with Na-clays containing highly hydrated Na ions. According to Wiegner (40) the ζ-potential of such a clay, as well as its degree of dissociation, must be high. The addition to the Na-clay of an electrolyte (NaOH) possessing a cation in common with the clay particle, must result in a shrinking of the double layer, i.e., in a decrease of the distance between the particles, and in flocculation (20). This, however, does not occur; on the contrary, an increase of solubility is observed. This increase of solubility can be most probably explained by the decrease of external friction between the lamellar particles of clay under the influence of the NaOH solution, a phenomenon established by Derjaguin for mica (7).

The arrangement of the experiments did not differ from the foregoing. Tenacious aggregates remaining after washing with distilled water were treated with a 0.05 N solution of NaOH. Washing with the same solution was continued until turbidity was no longer apparent above the sediment. After the alkali treatment, the sediment was washed with distilled water. All the clays generally produced some turbidity after the addition of distilled water. In some of them this turbidity was insignificant and disappeared quickly after washing. The shrinkage of the ion envelope under the influence of NaOH and the subsequent lessening of this shrinkage under the influence of distilled water seem actually to take place. The results of this experiment are summarized in table 3.

The experiments show that even treatment with 0.05N NaOH does not

destroy the aggregates entirely; on the contrary, the action of alkali on some clays is hardly different from that of pure water.

THE SOLUBILITY OF CA-CLAYS

From the viewpoint of the mechanism of coagulation and of the theory of ion exchange, the density of the ion envelope in Ca-clays occupies an intermediate position between highly hydrated monovalent ions and non-hydrated mono- and polyvalent ions. The value of the ζ-potential in Ca-clay is suffi-

TABLE 3

The influence of 0.05 N NaOH on the solubility of clay

CLAY	CLAY CONTENT OF THE RESIDUE AFTER WASHING WITH WATER (INSOLUBLE CLAY)	CLAY CONTENT IN THE RESIDUE AFTER TREATMENT WITH 0.05 *N* NaOH	LOSS
	*mgm.**	*mgm.**	*per cent*
1. Zhaki (loesslike loam, Würms)	129.1	117.6	8.9
2. Toida (cover clay, after Riss)	58.1	38.9	33.0
3. Kobeliaki (fluvio-glacial clay, Würms)	60.6	47.2	22.1
4. Ostrovy (bottom moraine, Riss)	68.3	†	†
5. Red clay (Carboniferous)	69.7	†	†
6. Alatyr (cover clay, after Riss)	67.7	62.3	8.1

* Per gm.

† Not measured.

TABLE 4

Comparison of the insolubility of Ca- and Na- clays

CLAY	Ca-CLAY CONTENT OF INITIAL MATERIAL	Ca-CLAY CONTENT OF RESIDUE AFTER WASHING WITH WATER (INSOLUBLE Ca-CLAY)	LOSS OF Ca-CLAY	INSOLUBLE Na-CLAY	LOSS OF Na-CLAY
	*mgm.**	*mgm.**	*per cent*	*mgm.**	*per cent*
1. Kobeliaki (fluvio-glacial clay)	111.6	61.8	44.6	60.6	58.6
2. Ostrovy (bottom moraine)	126.1	57.9	54.1	68.3	53.4
3. Red clay (Carboniferous)	152.4	109.4	28.2	69.7	43.1
4. Mokshany (cover clay, Riss)	99.6	83.2	16.5	....	....

* Per gm.

ciently high for the clay to be soluble. According to Baver (3) Ca-clay is even more hydrated than Li-clay. Our observations corroborate this assumption. Only the kinetics of this process are different: Ca-clay dissolves at a slower rate. Whereas in Na-clay the separation of the soluble from the insoluble fraction requires 3 to 5 days, in Ca-clay a slight turbidity is observed even after 15 days of washing. The results of our determinations are shown in table 4. Adsorbed Ca does not prevent the solution of clay. The percentage of Ca-clay lost during washing with water is almost the same as that of Na-clay. In some cases the solubility of Ca-clay even exceeds that of Na-clay.

Water acts more strongly on clay particles that are loosely packed and on particles irregularly distributed. They are protected from being washed off, not by absorbed calcium, but by Ca in the form of bicarbonate ions, as was long ago pointed out by Schloesing (35, p. 593): "La présence du calcaire n'aura pas préservé l'argile de l'entrainement, si le lavage a eu lieu avant que la végétation, source des débris qui fournissent l'acide carbonique et par suite le bicarbonate de chaux, se soit emparée du terrain."

ADSORPTION OF HUMATE ON QUARTZ SAND

The adsorption of humate on quartz sand, like that of clays, has not heretofore been studied. There seem to be no grounds for expecting that sand will adsorb humus, since quartz is an acid. On the other hand, experiments with the adsorption of organic substances on quartz have been described by many authors. The adsorption of proteins has been studied thoroughly (10). A marked alteration of the wetting ability occurs during the adsorption of hydrophobe substances—a fact of special interest (11). Recently the question has been studied in great detail by Lindau and Rhodius (22). They found that the adsorption of proteins on quartz follows the laws of Langmuir.

For the experiments with the adsorption of humates, the same quartz sand was used as for the experiments with clay. The volume of peat humate was 25–50 cc. for 5–10 gm. of sand. The time of adsorption was 1 hour. The experiments were arranged as previously described. Adsorption was determined by analyzing the equilibrium concentration of the humate. These experiments, repeated several times, showed no alteration in the equilibrium concentration. Following this experiment, one on the influence of drying was set up. Quartz powder, moistened with humate (concentration 1.92 gm. per liter) followed by elimination of excess liquid, so that only the adhesion rings remained, was dried at 35°C. The dried powder was washed thoroughly with water for several days. As there is no turbidity with humate, the washing was continued until the color of the sand remained unchanged. It was apparent, after this washing, that considerable quantities of humate adhere to the sand—1.58 mgm. per gram of sand. To determine the maximum amount of humate adhering to sand, a mixture of sand with peat humate, containing 12.08 per cent of humus, was prepared. The mixture was dried in the open at 35°C. then thoroughly washed with water. The amount of insoluble humus firmly adhering to sand was 5.8 mgm. per gram of the mixture.

Since the peat humate aggregates considerably less readily than do clays, one would expect that the mixture of sand and Ca-humate would prove more resistant to water than the mixture of sand and Na-humate. Indeed, the experiment showed that Ca-humate adheres to sand in greater amounts than does Na-humate. A mixture containing 12.5 per cent of Ca-humate was prepared, dried in the open, and thoroughly washed with distilled water until it ceased to lose humate. The amount of humus firmly adhering to the sand equaled 28.9 mgm. per gram of sand, consequently about 77 per cent of the

humus had been dissolved. Hence the organic substance of soil can be readily separated from sand, as has been indicated by Schloesing (35).

The situation is quite different in regard to the relation between humus and clay. As Schloesing says, ". . . l'argile est un ciment pour les éléments sableux de la terre, elle les relie et leur donne de la consistance" (35, p. 521), and further, ". . . le ciment minéral est, en quelque sorte, cimenté par le ciment organique" (35, p. 539).

ON THE BOND BETWEEN HUMUS AND CLAY

A certain amount of organic substance is most firmly bound with clay. A remarkable property acquired by clay under the influence of humus is the decrease of its solubility. Schloesing (35), on the basis of his observations, thought that "the colloids of humus take up clay, as it were, in a network thus robbing it of the capability to dissolve under the influence of water. . . . Clay carries away humate, while dissolved humate keeps clay back." Schloesing considered, therefore, that humus and clay form some "undefined compounds."

Recently the careful experiments of Tiulin (38) on the isolation of humates from organic-mineral gels showed that even with the aid of 0.05 *N* NaOH it is impossible to separate humus from clay completely.

Experiments performed in the course of the present work show that clay without previous drying is able to bind humate irreversibly. Thus there is a firm adsorptional bond between clay and humus. However, the accumulation of humus may be considerable, greatly exceeding the quantity necessary for the formation of the adsorption layer. A phenomenon similar to that in the case of clay-quartz sand mixtures takes place here. Mutual orientation, or the ability to form non-homogeneous aggregates, may be explained by the identity of the smectic phases of clay and humus, i.e., by their both being capable of mesomorphous aggregation. Rinne (32) is of the opinion, that "paracrystalline adsorption"[2] Phenomena extend not only to the group of regular intergrowths [i.e. those following the laws of Macles (13)], but to that of phenomena representing various kinds of stratification, such as the distribution of water and other substances in the zeolites.

For these experiments we prepared mixtures of humus with clay. In addition, we investigated the "organic-mineral" sediments after the separation of humates with a solution of KCl. The samples, according to the data of Tiulin, from whom the samples were obtained, contain much humus bound with clay. It is possible, however, to separate some humus by treatment with 0.05 *N* NaOH. As a result, clay is obtained containing almost completely insoluble humus. Such clays were also tested. In some cases a remarkable picture of dendritic-like branchings, indicating a rapid growth of crystals, may be observed under the microscope with crossed Nicol prisms. The structure obtained reminds one only remotely of the structure of the original

[2] Rinne uses the term "paracrystalline" instead of "mesomorphous" to emphasize the natural close relationship between the conceptions of "crystal" and of "paracrystal."

material (pl. 2, figs. 2, 3, 4). It is possible to see the dendritic branches radiating from a common center and to produce, in the same way, spherulites in parallel light with crossed Nicol prisms, the dark cross of the single-axis crystal keeping its position unchanged at the rotation of the stage of the microscope. The cause of this is the radial disposition of the filaments in respect to the center, readily visible in figure 4, plate 2. In the lucid quadrants of the spherulite cross in a gypsum plate of the first order it is possible to establish the negative optical character of the formation. In some parts of the preparation the transformation of the negative sign of double refraction into the positive one may be observed. According to Vorländer (39) this alteration of the optical character is related to the different arrangement of the molecules in the elementary cell. In gypsum of the first order a remarkably beautiful coloring of the different sections of the preparation and the mutually opposed transition of the colors at the rotation of the microscope stage, indicating twinning, are observed.

The structures represented in figure 5 (prevalence of humus) and figure 16 (prevalence of clay), plate 2, are characteristic of most clay-humus mixtures. As a rule, humus decreases the double refraction of clay. No sign change is observed here. Spherulites, not observed in clay, appear in the mixtures and show, in covergent light, the dark cross of one-axis crystals. The characteristic "oily strip" structure is transformed into a fluid one (pl. 2, fig. 6). The decrease of double refraction is observed also in clay samples which were allowed to swell in humate solutions. "Grade drops" ("gouttes à gradins" of Grandjean, 12) are visible in natural light in the "hollows" between the noticeably twinned smectic lamellas (pl. 2, fig. 7).

It is hardly possible to establish the distinctive features characterizing mixtures with a greater or lesser humus content. "Stratification" of humus on, or its "intergrowth" with, clay proceeds to a certain limit, and excess humate does not affect the structure of the mixture. This is suggested by a comparison of the structures observed in the sediments isolated from soil and representing an "inseparable mixture" of humus and clay with those observed in artificially prepared mixtures. Figure 9, plate 2, shows the structure in natural light of such an "inseparable sediment" of clay with humus, isolated from soil; figure 10 shows the same preparation in polarized light; and figure 11, the structure of an artificial mixture of clay with humus. Figure 11, plate 2, shows excess organic substance being drawn out to the periphery by a stronger tension than that in the clay surface (31). Only the most external rows of micellae form the smectic phase. Close to this oriented layer lies a zone showing no double refraction (amorphous organic substance). Inside of the amorphous layer lies the smectic substance with a dendrite structure, which represents the "inseparable" combination of clay and humus. The amorphous organic substance is readily dissolved as soon as the layer of oriented particles surrounding it is destroyed.

The sediments isolated from soil and representing an inseparable combination of humus and clay have a different structure from that of mixtures in that

here there is no amorphous readily dissolving layer of organic substance at the periphery. Here the smectic substance of a dendrite structure is surrounded by a zone of intense double refraction (pl. 2, fig. 10).

DECREASE OF THE SOLUBILITY OF CLAY UNDER THE INFLUENCE OF HUMUS

Many authors have expressed their opinion on the protective rôle of humus (30), but no adequate theory of this phenomenon has been supplied. The protective influence of humus was considered as hindering the coagulation of mineral suspensions. Buzagh (4) has discovered the stabilizing effect of humus on kaolin suspensions. The explanation of these phenomena is possibly connected with the alteration of the moisture capacity under the influence of humate adsorption. The protective effect of proteins on bentonite suspensions in the experiments of Ghosh (15) may be explained by a transformation of the hydrophilic surface of bentonite into a hydrophobic one. Bartell and Walton

TABLE 5

Accumulation of clay by repeated condensation

CLAY	CLAY CONTENT IN INITIAL SAMPLE a	CLAY CONTENT AFTER WASHING WITH WATER a_1	LOSS	
	*mgm.**	*mgm.**	*mgm.**	*per cent*
1. Dzekan	20.64	3.63	17.01	82.4
2. Red clay	8.5	0.86	7.64	90.0
3. Toida	21.41	4.58	16.83	78.6
4. Kobeliaki	23.73	3.00	20.73	87.4
5. Ostrovy	12.37	....	.....	....
6. Mokshany	13.49	0.72	12.77	94.6

* Per gm.

(2) point to the possibility of an arbitrary alteration of the wetting of the same surface. There are some observations the extension of which to humus seems justifiable in a certain measure. Thus, in the experiments of Kruyt (21) a tannin film transforms many hydrophilic colloids into hydrophobic ones. Kruyt affirms, that the molecules of tannin may be regarded as polar ones, and they orient themselves in such a manner that their hydrophobic ends are turned in the direction of water. According to the data of Lindau and Rhodius (22) the moisture capacity of quartz powder decreases according to the amount of albumin covering the powder, and when the albumin reaches 50 per cent the powder rapidly passes from the hydrophilic into the hydrophobic condition.

The purpose of our experiments was to establish the decrease of the solubility of clay pellicles on quartz sand under the influence of humus. The most unfavorable conditions in respect of the solubility of clay envelopes on sand were chosen for these experiments: thick, readily soluble envelopes were prepared. As in the preceding experiments, Na-clay was used. Quartz sand

moistened with the clay suspension was dried at 35°C. after the elimination of excess liquid, moistened with a clay sol, and again dried. This manipulation was repeated several times, and, indeed, as the determinations of the amount of clay have shown (table 5) the envelopes obtained were much thicker than those of the first experiment (table 1). It was found that simultaneously the amount of clay firmly bound to the sand (insoluble clay) had also increased. As may be seen from these figures the sand contained readily peptizable clay. The experiments for the determination of the influence of humus on the decrease of the solubility of clay were made with this sand.

The volume of the humate solution equaled 60 cc. for 6 gm. of the sample, calculated on the basis of absolutely dry weight. The time of adsorption was 1 hour, during which the sand was thoroughly mixed with the humate solution. After this, the liquid was filtered through a filter which retained all the sand. The concentration of the equilibrium solution (C), the adsorption of humate,

TABLE 6

Reciprocal relation of the adsorption of humus and clay

CLAY	C	HUMATE ADSORBED	AMOUNT OF CLAY DISSOLVED IN HUMATE	REMAINING CLAY, ABLE TO PEPTIZE IN WATER
	per cent	*mgm.**	*mgm.**	*mgm.**
1. Dzekan	0.192	0	2.08	14.93
2. Red clay	0.186	0.22	2.08	5.56
3. Toida	0.190	0.08	0.56	16.27
4. Kobeliaki	0.192	0	2.08	18.65
5. Ostrovy	0.188	0.15	3.12	.....
6. Mokshany	0.185	0.29	0.32	12.45

* Per gm.

and the amount of clay which had been dissolved in the humate were then determined (table 6).

The adsorption of humate by clay, generally only an insignificant value, was not observed at all in some of the clays. Adsorptional layers hardly reach the values necessary for even a monomolecular layer. Accordingly the determination of the decrease of the solubility of clay under the influence of freshly deposited humate was not undertaken. We decided to repeat this experiment using a thicker layer of clay. To form a more compact layer of humate on the clay envelopes, the sand, remaining after the adsorption experiment, was freed from excess humate solution by suction with a water-pump and then dried at 35°C. The dried sand was again moistened with humate and again dried, i.e., the same manipulations as with clay were repeated with humate. Thus the clay layer able to peptize in water was "protected" by a thicker layer of humus. The decrease of solubility is easy to determine if one compares the amount of soluble clay (table 6) with the amount remaining on

quartz after washing with water (table 7). The sand, covered with clay and humus, was washed with water according to the technique previously described. The dissolved humate was determined by difference: the initial material and after it had been washed with water. The dissolved clay was determined by the amount remaining after washing. The remaining insoluble clay was determined, as before, by dissolving in HCl solution. A necessary correction was made in the value obtained to allow for the humus contained in the insoluble clay.

The results of these experiments show that the solubility of clay is decreased under the influence of insignificant amounts of humus—and that drying increases the irreversibility of the humate. A possible explanation of this is the formation of an adhesion layer and the orientation of the molecules at the surface of quartz sand mixed with clay.

TABLE 7

The influence of humus on the decrease of the solubility of clay

CLAY	HUMUS CONTENT OF INITIAL MATERIAL	HUMUS CONTENT AFTER WASHING WITH WATER	CLAY CONTENT AFTER WASHING WITH WATER (IRREVERSIBLE CLAY)*	LOSS		DECREASE OF SOLUBILITY
	*mgm.**	*mgm.**	*mgm.**	*mgm.*	*per cent*	*per cent*
1. Dzekan	2.9	2.55	5.18	10.75	67.4	15.0
2. Red clay	2.0	1.74	2.15	3.41	61.3	28.7
3. Toida	2.04	1.63	10.38	5.89	36.2	12.4
4. Kobeliaki	3.34	1.0	11.13	7.52	40.3	47.1
5. Ostrovy	4.20	0.65	11.7	.....	....	....
6. Mokshany	4.40	2.0	1.53	10.92	87.9	6.7

* Per gm.

DECREASE OF CLAY SOLUBILITY UNDER THE INFLUENCE OF FRESHLY DEPOSITED LAYERS OF HUMATE

Samples of quartz sand-Na-clay mixtures which had been used for the study of the tenacity of the bond between Na-clay and quartz sand (table 2) were taken for these experiments. The phenomenon of the adsorption of humate should be still more evident in this case because of the greater content of clay. The volume of humate used was 60 cc. for 1 gm. (absolute dry weight). The time of adsorption was 1 hour, during which the material was mixed several times. After this, the liquid was filtered, and the concentration of the equilibrium solution was determined. The residue was washed with water immediately after filtration until no turbidity appeared in the filtrate. After the washing was completed, the sediment was dried and the content of undissolved (irreversible) humus and that of undissolved (irreversible) clay were determined. The results are summarized in table 8.

The decrease of the solubility of clay is related to the adsorption of humus but does not show an equal correlation with it in all the tested samples of clay.

Apparently the mineralogical composition and the influence of contaminations play a certain part in this matter. In some cases the adsorption of humate produces no change in the solubility of clay (clay "Zhaki") or only slight changes.

INFLUENCE OF DRYING

The experiment was modified in comparison with the preceding experiment. The samples used were the same, but the saturation with humate was brought about by letting the material swell in humate solution. The samples were

TABLE 8

Adsorption of humus on clay

CLAY	HUMUS ADSORBED	IRREVERSIBLE HUMUS ADSORBED	CLAY CONTENT AFTER WASHING WITH WATER (IRREVERSIBLE CLAY)*	LOSS	DECREASE OF SOLUBILITY
	*mgm.**	*mgm.**	*mgm.*	*per cent*	*per cent*
1. Zhaki	1.83	1.64	117.6	18.0	0
2. Toida	....	1.64	96.0	36.6	25.0
3. Kobeliaki	1.59	1.50	68.7	53.6	5.6
4. Ostrovy	1.34	0.98	80.0	45.5	7.9
5. Red clay	1.27	0.92	....	....	....
6. Alatyr	1.16	0.91	103.9	26.2	25.7

* Per gm. of mixture.

TABLE 9

Influence of humus drying on the solubility of Na-clay, mixed with sand

CLAY	HUMUS CONTENT IN INITIAL MATERIAL	IRREVERSIBLE HUMUS	IRREVERSIBLE CLAY	LOSS OF CLAY	DECREASE OF SOLUBILITY
	*mgm.**	*mgm.**	*mgm.**	*per cent*	*per cent*
1. Zhaki	12.8	6.1	118.4	17.1	0
2. Toida	15.1	7.4	118.8	21.5	36.6
3. Ostrovy	9.5	9.3	107.4	27.0	41.3

* Per gm.

placed on glass slides covered with filtering paper, the ends of which were immersed in a humate solution. As the evaporation from the surface of the samples was not eliminated (the container with the swelling samples was placed in a large crystallizer covered with filter paper to protect it from dust) the saturation with humate constantly increased. After 15 days the supply of humate was removed and the samples were left to dry slowly at room temperature. All the samples during the swelling showed a fine structure with horizontal fissures forming a number of parallel lamellas. In drying, these samples acquired the ability to fall into small clumps, reminding one by their

shape of the natural structure of soil, unlike the viscous consistence of the samples of sand—Na-clay mixtures. Moreover, these clumps proved tenacious and water-tight. Otherwise, the study of these samples differed in no way from the preceding. The samples were treated with water to eliminate all soluble matter (i.e., clay and humus). After this washing, the remaining humus and clay were determined, and their amounts were compared with those in the initial material (table 9).

Drying increases the accumulation of humus in an irreversible condition. Simultaneously, the solubility of clay is further decreased. Again the fact stands out that on some clays, humus has no effect.

DISCUSSION OF RESULTS

The drawing together of the colloidal particles, which occurs during the formation of the phases of the aggregate state, leads to a deformation of the ion envelope around these colloidal particles. This deformation is expressed in a compression of the ion layer or in a decrease of the distance of the double layer, and must affect all the properties, depending on the dimension of the double electric layer. The density increase of the ion layer decreases the value of the ζ-potential, decreases the degree of dissociation, and consequently must decrease the solubility and exchange capacity.

In our experiments the decrease of the solubility of Na-clay mixed with sand and humus depends on the compactness of the particles. A homogeneous arrangement of the particles corresponds to the most compact condition and, therefore, is the most stable one. An accumulation of irregularly arranged particles and a loose (non-homogeneously orientated) packing represent an unstable system, the properties of which depend on the dimensions of the ion layer. The conceptions relating to the theory of the colloidal micella as a surface with a double electric layer are essential for the explanation of the properties of this system. The properties of such an unstable system depend in a high measure on the nature of the absorbed bases. The shrinking of the ion envelope at the grouping of the particles levels out the difference due to the kind of absorbed cation, and explains the equal solubility of the aggregates prepared from Na-clay and from Ca-clay. Hence the notions existing in soil science in regard to the behavior of soil colloids proved to be only partly correct, since they do not take into account the ability of soil colloids to form phases of the aggregate state. The instability of Na-clay suspensions is also easily explained by the deformation of the ion envelope at the association of particles into groups. According to Jenny, the curve representing the ratio between the ζ-potential and flocculation is in the form of a parabola, showing that the stability of a clay suspension increases several times at even a slight rise of the ζ-potential. The suspension proves stable only within a narrow interval of the ζ-potential, ranging between 60 and 40 m.v. At ζ-potential = 40 m.v., clay coagulates in the absence of electrolytes. This tendency of clay suspensions to flocculate is explained by the ability of clays to form phases of the

aggregate condition. Because of this, clay coagulates more readily than humus, of which the capability to aggregate is much weaker.

The depression of the ion layer in the aggregated particles results also in a decrease of the exchange capacity. This decrease is always observed during the determination of the absorbing capacity of stable aggregates. According to the data of Tiulin the absorbing capacity of the so-called "isoelectric group of gels" is always lower than that of the first fraction of "electronegative" gels. In the same measure, the decrease of the absorbing capacity is characteristic of the so-called "passive" slime of Sokolovsky.

The cohesion between the individual parts of a non-homogeneous aggregate consisting of colloidal particles and of particles of a greater size may be explained by the orientation of the molecules of the layer of adsorption only in the limited case of the closest contact between the coarse mineral elements. In most cases the orientation of the molecules of the layer of the cementing substances extends to a certain depth, there to become disorganized and to be replaced by the smectic elements of the grouping. The increase of the stability of the bond and the decrease of the solubility of the clays cannot be explained only by the influence of the adsorbed layer of humus and by the decrease of moisture capacity due to a definite orientation, as is recognized, for instance, in regard to the molecules of fatty acids. In soil we are dealing with a large accumulation of humus in an irreversible state and with the prevalence of the variable forms of the mesomorphous grouping.

It is striking, indeed, that there can be highly stable (i.e., insoluble in water) non-homogeneous aggregates composed of parts which are separately readily soluble in water. The sol of Na-clay after it has been dried at 100°C. readily dissolves again in water, but, mixed with pure quartz sand, it becomes insoluble and acquires the character of a very tenacious cement. A similar behavior is manifested by humus-clay aggregates obtained from a Na-clay and Na-humate material, which readily peptizes under the influence of water. These aggregates acquire a remarkable resistance not only to water, but also to alkalis.

SUMMARY

The former conception that the particles forming the soil structural aggregate are held together by the tension of the water film is supplemented by the finding that the surface film creates a definite orientation of the particles in relation to one another.

The tenacity of the bond between the separate parts of the soil structural aggregate cannot be explained by the presence of absorbed Ca. The explanation of the tenacity of this bond lies in the stability of the group arrangement of the particles. The most stable arrangement of particles is the homogeneous one.

The formation of non-homogeneous aggregates in soil—sand-clay and clay-humus—may be explained by the same laws which have been found to exist for mineral intergrowths.

The impossibility of completely separating soil into its elementary parts is corroborated. In connection with the theory developed, it becomes necessary to revise the theory of soil texture. The subdivision of soil into stable and unstable groupings of particles appears to be more expedient.

The influence of external conditions (vegetation, microorganisms, tillage, pressure, freezing, etc.) on the formation of structure can be explained by the swarm theory.

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PLATE 2

Microphotographs of Clay-Sand and Humus-Clay Mixtures

Fig. 1. Clay-sand mixture in polarized light. Magnification ×3.

Fig. 2. Humus-clay mixture. Dendrites. Polarized light. Magnification ×40.

Fig. 3. Another position of the preparation shown in figure 2. Magnification ×40.

Fig. 4. Another position of the preparation shown in figure 2. Spherulite of a mono-axis crystal. Polarized light. Magnification ×40.

Fig. 5. Humus-clay mixture. Non-homogeneous structure. Oily stripes and spherulites of a mono-axis crystal. Polarized light. Magnification ×27.

Fig. 6. Humus-clay mixture. Fluidal structure. Polarized light. Magnification ×27.

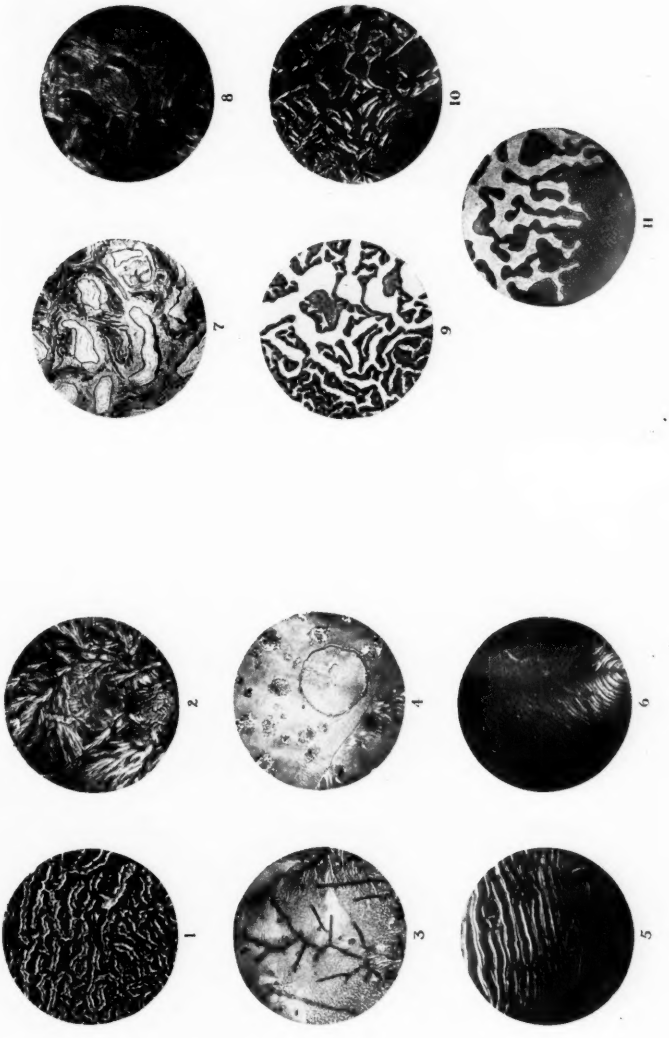
Fig. 7. Clay "Toida." Swelling in humus. "Grade drops" of Grandjean. Natural light. Magnification ×27.

Fig. 8. The preparation shown in figure 7 in polarized light. Magnification ×27.

Fig. 9. Structure of "inseparable" humus-clay precipitate. Natural light. Magnification ×27.

Fig. 10. The preparation shown in figure 9 in polarized light. Magnification ×27.

Fig. 11. Artificial mixture of humus with clay. Natural light. Magnification ×27.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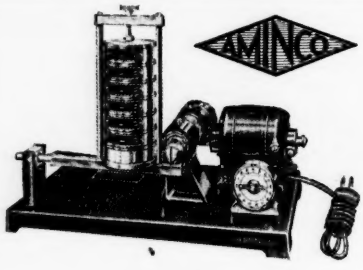

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